



Electrochemistry in a centrifugal microfluidic system

Towards a novel point-of-care technology platform

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Development of Coenzyme Q10-Based Electrochemical Sensors for the Detection and Quantification of Acetylcholine and other Tetrasubstituted Amines

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This work presents advances towards the development of a sensor for acetylcholine and other tetrasubstituted amines based on the enhanced reduction of Coenzyme Q10 in lipid membranes when these molecules are present. We have previously shown that the electrochemistry of Coenzyme Q10 incorporated into lipid bilayers is strongly affected by the presence of hydrophobic tetrasubstituted amine cations in the aqueous environment. The reduction of Q10 is facilitated in these media, resulting in a quantifiable positive shift in the mid-peak potential of the reaction as recorded using cyclic voltammetry (CV). This shift is in the order of hundreds of mV and is unique for each cation, allowing for qualitative determinations. It is proposed that the shift is caused by ion-pair formation between the produced semiquinone anion and the studied cation, as illustrated in the figure below. Furthermore, the peak current intensity in CV experiments is dependent on the concentration of the cation in the solution, and can therefore be used for quantitative analyses. Remarkably, this technique can be employed to detect and quantify acetylcholine, and could therefore be useful for the early diagnosis of neurodegenerative illnesses such as Alzheimer's disease. Q10 can be incorporated into supported lipid bilayers, immobilized liposome layers, and immobilized PEG-stabilized lipid bilayer disks (lipodisks), providing thus a wide range of possibilities for future applications. Furthermore, the electrochemical measurements can be coupled with quartz crystal microbalance with dissipation monitoring (QCM-D) experiments, improving the sensitivity and specificity of the technique.

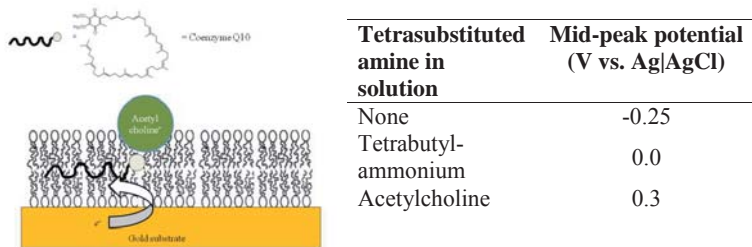


Figure: Schematic representation of the ion-pairing reaction coupled with the electrochemical reduction of Coenzyme Q10 embedded in a lipid membrane. The table to the right illustrates the mid-peak potentials obtained with different tetrasubstituted amines. All data was obtained at pH = 7.4.

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The unexpected activity of Pd nanoparticles prepared within a non-ionic surfactant template

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Abstract

Pd deposits on vitreous carbon substrates were prepared by electrodeposition from liquid crystal phases (both micellar and hexagonal phases) consisting of self-assembled non-ionic surfactant molecules. The morphology of the deposits varied significantly with the concentration of surfactant but all are made up of aggregated nanoparticles circa 9 nm in diameter. The deposits from the micellar phase of the surfactant offer the largest electroactive area and specific activity for the hydrogen evolution, oxygen evolution and reduction reactions, formic acid and ethanol oxidations. Unexpectedly the deposits yield an increase in catalytic activity far in excess of that expected from an enhancement in electroactive area.

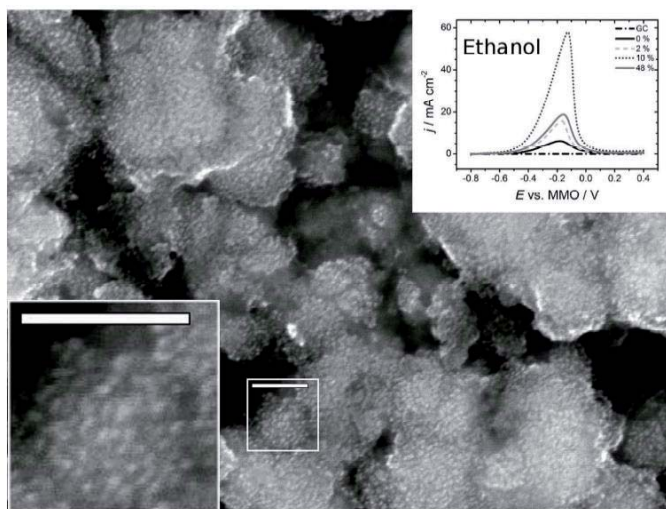


Figure 1 FEG-SEM image of Pd structure electrodeposited on glassy carbon (GC) after stepping from +0.7 V to -0.3 V vs. SCE for a 63.7 mC cm^{-2} deposition charge. 10 wt. % surfactant. The scale bar is 100 nm long. Inset figure shows Voltammograms for the oxidation of ethanol recorded in Ar purged 0.5 M ethanol + 0.1 M KOH at 100 mV s^{-1} with GC electrodes decorated with the Pd deposits. The legend indicates the corresponding plating mixtures.

Conducting 3D-carbon scaffolds induce spontaneous differentiation of human neural stem cells and measure neurotransmitter release in real-time

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Conductive three-dimensional (3D) scaffolds show promise for sensing of cellular differentiation and/or electrical stimulation. Here, structurally patterned pyrolysed 3D-carbon scaffolds (p3D-carbon) were fabricated and applied for differentiation and sensing of neurotransmitters released from human neural stem cells (hNSCs) developed for cell replacement therapy. The pyrolysed carbon material induced spontaneous hNSC-differentiation into mature dopamine-producing neurons and the 3D-topography promoted neurite elongation. Depending on the conditions, ~73-82 % of the hNSCs obtained dopaminergic properties on pyrolysed carbon, a to date unseen efficiency in both 2D and 3D environment. Due to conductive properties and 3D-topography, the p3D-carbon served as a neurotransmitter trap, enabling electrochemical detection of a significantly larger dopamine fraction released by the hNSC-derived neurons than on planar pyrolysed carbon. This is the first study of its kind, presenting new conductive 3D scaffolds which provide highly efficient hNSC-differentiation combined with real-time in-situ confirmation of the fate of the hNSC-derived neurons.

Electrochemistry in a centrifugal microfluidic system: Towards a novel point-of-care technology platform

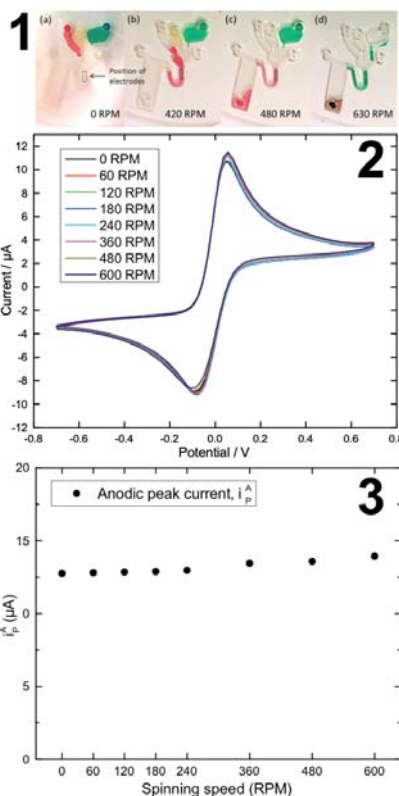
Sune Z. Andreasen, Dorota Kwasny, Anna Line Brøgger, Filippo G. Bosco, Karsten B. Andersen, Winnie E. Svendsen, Jenny Emnéus and Anja Boisen

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The Lab-on-a-Disc, or centrifugal microfluidic platform, has attracted a lot of attention in the last twenty years, and with good reason, as the principle of the inherent centrifugal pumping and small footprint gives the prospect of a simple-to use platform suitable for point-of-care (POC) devices. Typically Lab-on-a-Chip systems are based on external pumping, whereas Lab-on-a-Disc platforms are designed for handling liquids at the micro- and nanoscale by simply employing centrifugal forces, generated by rotating the microfluidic disc at given rotational speeds [1].

Here we present a centrifugally driven microfluidic system with integrated electrochemical sensing. The connection between the potentiostat and the microelectrodes on the disc is made by combining a commercially available electrical swivel with a custommade plug. The microfluidic system (Fig. 1) is designed with three capillary burst valves in parallel, so that different solutions can be added to the electrochemical cell continuously simply by increasing the spin-rate, and without the electrochemical cell being exposed to air between addition of the next solvent/solution, (Figure 1b-1d).

The behavior of the electrochemical system has been evaluated using cyclic voltammetry, both in static mode and during centrifugation. The voltammograms showed quasi-reversible behaviour in all conditions and absence of noise when rotation was applied (Figure 2 and 3). These results demonstrates the possibility of combining advanced pump-free liquid sample handling with sensitive electrochemical detection to achieve fully-automated sample processing based on an electrochemical read-out.



1 Demonstration of the centrifugal microfluidic sample handling using fruitcolored water. **2** Overlay of cyclic voltammograms (10 mM ferro-/ferricyanide in PBS, scan-rate: 0.2 V/s, potential vs Au) obtained while the disc was spinning. **3** Plot of the measured anodic peak currents as a function of spinning speed of the centrifugal platform.

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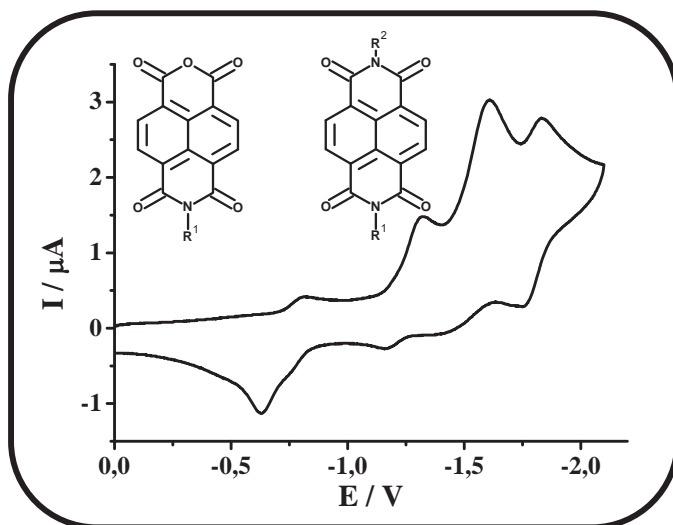
Novel Multielectron Transfer Photosensitizers Based on Chiral Imides: Their Photophysical and Electrochemical Properties

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There is a growing interest in the design of efficient multielectron transfer photosensitizers in the field of electrocatalysis, coordination compound research, fuel cell applications, biomimetic chemistry and chemical conversion of solar energy [1]. On the other hand, 1,4,5,8-naphthalene-diimide and monoimide derivatives are effective reagents in the formation of Langmuir-Blodgett films, the preparation of electrically conducting materials, π -stacked materials, nanotube-like structures and the models for the photosynthetic reaction center [2].



In this study chiral naphthalene imides with multielectron transfer properties have been synthesized. The compounds characterized by UV-Vis, IR, Fluorescence Spectroscopy, NMR, DSC, TGA and CV measurements. The optical, photochemical, thermal and electrochemical properties of the compounds have been investigated for the use in a number of different hi-tech applications such as photo-, electro-, and optoelectronic applications.

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Synthesis and Characterization of Modified Fe₃O₄ Magnetic Nanoparticles with ZrO₂ and its Application in Indirect Electrochemical Detection of Cr (III) and Cr (VI)

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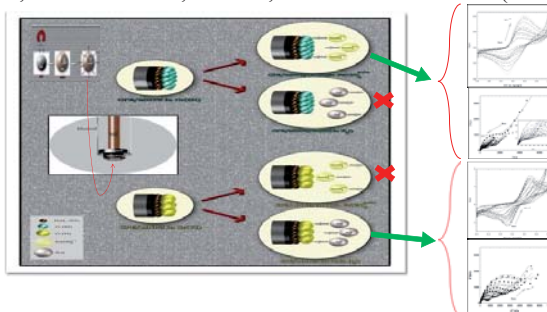
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Abstract

Magnetic nanoparticles are of great interest for researchers from a wide range of disciplines, including magnetic fluids, catalysis, biotechnology/biomedicine, magnetic resonance imaging, data storage, and analytical applications [1-2]. Recently, successful application of such magnetic nanoparticles in the mentioned areas is highly dependent on the appropriate surface engineering to ensure achieving desirable interactions with analyte. To this end, magnetite nanocrystals have been coated with different materials like hydrophilic polymers or inorganic shells [2-3]. Herein, modified Fe₃O₄ magnetic nanoparticles (NPs) by zirconium oxide (ZrO₂) were prepared (Fe₃O₄/ZrO₂). Prepared Fe₃O₄/ZrO₂ NPs were characterized with XRD, SEM, EDXA, TEM, FTIR and VSM techniques. In addition, by using a surface modified magnetic carbon paste electrode, interaction of Fe₃O₄/ZrO₂ NPs with Cr (III) and (VI) ions was investigated in the presence of Fe(CN)₆]^{3-/4-} and PBQ/H₂Q, as a probes. Electrochemical studies showed, on can detect each form of the chromium ions in the presence of another by controlling the pH and choosing appropriate redox probe via cyclic voltammetry (CV) or electrochemical impedance spectroscopy (EIS) methods. Our experimental results showed Cr (III) and Cr (VI) can pre-concentrate from 10⁻⁹ to 10⁻³ M in pH 3.5 and pH 4.0 and determined in the presence of Fe(CN)₆]^{3-/4-} and PBQ/H₂Q as a redox probe, respectively. Detection limits as 8.1×10⁻¹⁰ and 6.9×10⁻¹¹ M were observed for determination of Cr (III) by using CV and EIS techniques, respectively. In addition, detection limits as 1.4×10⁻⁸ and 4.5×10⁻¹⁰ M were observed for determination of Cr (VI) by using CV and EIS techniques, respectively. Observed results will be presented and discussed.

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Silver amalgam electrodes in environmental electroanalysis

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Up to now the best electrode material for cathodic reductions is mercury. However, somewhat unsubstantiated fears of mercury toxicity and connected legal obstacles with its use in analytical laboratories, prompted us to pay attention to various forms of amalgam electrodes [1,2] which present green and non-toxic alternative to dropping or hanging mercury drop electrode. Their possibilities and limitations for the determination of electrochemically reducible biologically active organic compounds in various environmental and biological matrices will be presented. Advantages of mercury meniscus modified silver solid amalgam electrode (m-AgSAE) will be demonstrated on voltammetric determination of insecticide Thiamethoxam in nanomolar concentration range [3] and carcinogenic 2-nitrobiphenyl and 4-nitrobiphenyl in submicromolar concentration range [4]. Environmental application of single crystal solid amalgam electrode [5], silver amalgam paste electrode [6] and silver amalgam tubular detector [7] will be discussed together with possibilities of silver solid amalgam electrode modified by a microcrystalline natural graphite–polystyrene composite film [8] or bismuth film [9]. Further possibilities of amalgam electrodes including their miniaturization, chemical and biological modification and application in flow systems will be outlined.

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Interdigitated ring electrodes: Theory and Experiment

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The oxidation of potassium ferrocyanide, $K_4Fe(CN)_6$, in aqueous solution under fully supported conditions is carried out at interdigitated band and ring electrode arrays, and compared to theoretical models developed to simulate the processes. Simulated data are found to fit well with experimental results using literature values of diffusion coefficients for $Fe(CN)_6^{4-}$ and $Fe(CN)_6^{3-}$. The theoretical models are used to compare responses from interdigitated band and ring arrays, and the size of ring array required to approximate the response to a linear band array is investigated. An equation is developed for the radius of ring required for a pair of electrodes in a ring array to give a result with 5% of a pair of electrodes in a band array. This equation is found to be independent of the scan rate used over six orders of magnitude.

This work has been published in the *Journal of Electroanalytical Chemistry*, 709 (2013) 57.

Characterisation of an Electrode with Immobilized Recombinant Protein for the Rapid Detection of Oestrogen

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We have previously established the detection of oestrogen using *Candida albicans* and *Saccharomyces cerevisiae* cells [1] and cell lysate [2].

Here we describe the strategy used to clone an oEstrogen binding protein sequence (EBP) based on the *Candida albicans* gene (accession number P43084) into non-pathogenic yeast for safe production of the protein. The gene was sequenced in 1991 by researchers interested in the pathogenicity of *C. albicans* [3]. The sequence was modified to meet the sequence preferences of the host, *Arxula adeninivorans* and by the addition of a 6-histidine tag (histag). EBP was purified by mechanical disruption of the cells, passing the crude lysate through a Ni-sepharose column and releasing the bound protein with imidazole. The identity of the purified protein was confirmed by amino acid sequencing and by western blot. The protein was initially used in solution but was subsequently immobilised onto the surface of a SPCE.

Immobilisation of the protein onto the surface a disposable carbon electrode was achieved by two methods. First, the protein was cross-linked to an unmodified carbon surface of a 'DropSens' screen-printed electrode (DropSense DRP-110) using glutaraldehyde. The second method exploited the terminal his-tag to bind the protein to a nickel-modified carbon electrode (DropSense DRP-110NI). Ethanol and protease were used to confirm the immobilization and AFM was used to visualise the presence of EBP on the electrode surface.

Detection of the EBP response to oestrogen involved the use of 2,3,5,6-tetramethyl-1,4-phenylenediamine (2,3,5,6 TMPD) to transfer electrons from the redox site of EBP to the electrode and incorporation of a mediator into the electrode so that it would interact with EBP after the sample is applied will described. Differential pulse voltammetry was used to quantify the response to oestrogens.

The modified SPCEs were freeze dried and stored at 4 °C in air until use. Electrode characteristics including limit of detection and determination, substrate range, pH range, temperature range, response time and stability were established.

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On-line monitoring of trace antimony, arsenic, cadmium, cobalt, copper, and thallium by flow-through stripping chronopotentiometry in metallurgical concentrated zinc and cadmium solutions

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The production of zinc through the electrowinning technology is mostly based on the electrolytical deposition of metallic zinc from concentrated zinc sulphate solutions. The quality of the product and electrolytical yield are influenced considerably by the content of other metals in the solution, such as antimony, cobalt, copper, cadmium, and thallium. Their concentration has to be measured, preferably on-line, to ensure a proper control of the technology.

The roasting process of sulfidic zinc ores may produce large amounts of cadmium vapours as well which are then converted to concentrated cadmium solutions. Cadmium metal is extracted through chemical reduction by zinc powder in acidic media. The procedure is extremely dangerous if the cadmium solutions contain also arsenic which may be converted to poisonous arsine gas during the reduction step. A reliable control of the arsenic concentration, again in on-line mode, is therefore inevitable to make the technology safe.

Both tasks have been solved by making use of an analytical procedure based on stripping chronopotentiometry in flow-through mode. The analysed concentrated zinc sulphate solutions with zinc contents up to 160 g/L were 10-times diluted on-line first then analysed in the electrochemical system. Co, Cu and Cd were measured in the sub-mg/L range, Tl in the 0.1 – 50 mg/L and Sb in the 1 – 20 µg/L ranges, respectively.

The cadmium sulphate solutions contained up to 50 g/L Cd which obviously interfered at the electrochemical arsenic determination. The interfering effect of cadmium ions was eliminated by on-line removal of cadmium in a column packed with a strong cation exchanger. Arsenic contents down to few µg/L could then be measured. On eluting the trapped cadmium from the column it was possible to determine the cadmium concentration as well by making use of the same electrochemical system.

The results have fully confirmed the suitability of electrochemical methods for such types of samples and species which could hardly be monitored by other methods. Technical details about the fully automatic sample preparation and electrochemical systems will be presented in the paper together with measured and reference data the latter delivered by atomic spectroscopy.

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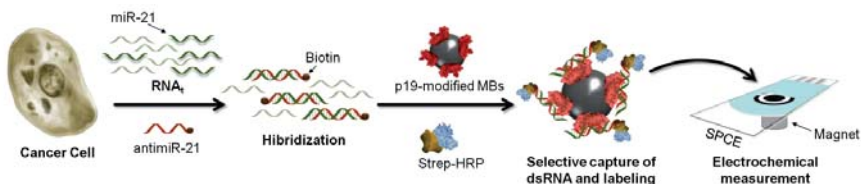
Viral protein p19-based magnetobiosensors for miRs determination in cancer cells

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MicroRNAs (miRs) represent a class of minimally invasive biomarkers which have shown a great promise for cancer diagnosis, progression, prognosis and response to treatment. However, several intrinsic characteristics of miRs (short sequence length, low abundance and high sequence similarity) make their detection challenging. Currently, miRs are detected by expensive, complicated and time consuming techniques such as Northern blot, RT-PCR, and microarrays which are not feasible for on-site determinations. Therefore, there is an urgent need to develop novel methods designed to measure miRs in a reliable manner with high specificity and sensitivity. In this sense, electrochemical nucleic acid biosensors are particularly attractive and emerging options in terms of their ease of use and automation, low assay time, low detection limit, small amount of sample required and non-toxic experimental steps.

Here, we report for the first time amperometric magnetobiosensors involving RNA binding viral protein p19, as highly selective biorecognition element, for miRs quantification (see Scheme 1).



Scheme 1.- Schematic display of the p19-based amperometric magnetosensor designed for the determination of miR-21.

The p19-based magnetosensors were able to detect, in only 2 h, 0.4 fmol of synthetic target and endogenous miR-21 (selected as model for its role in a wide variety of cancers) directly in total RNA extracted from cancer cells without PCR target amplification or labelling and sample pre-processing. The magnetobiosensor avoids the needs for complicated temperature control (which provided significant potential applications in clinical analysis) and features the convenience of commercial screen-printed based electrochemical sensing. This strategy is envisaged to open a new venue for large-scale production of disposable one-shot miRs diagnostics, particularly feasible for routine detection in both clinical and research settings.

Extracellular Polymeric Substances in Anode Biofilms of Microbial Fuel Cells

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Extracellular polymeric substances (EPS) are high-molecular weight compounds from microorganisms which are typically composed of polysaccharides, proteins, nucleic acids, lipids, and present both outside of microorganisms and in the interior of microbial aggregates and biofilms. Electroactive microbial biofilms have ability to transfer electrons from organic compounds to anodes in microbial fuel cells (MFCs). Investigating the composition and distribution of EPS in anode biofilms is important for enhancing our understanding of anodic biofilm formation. In this study, EPS composition was analyzed using a mixed bacterial biofilm on carbon fiber cloth anode with high power generating ability in single chamber MFCs. Single-chamber membrane-free MFCs (12 ml) were constructed as reported previously. The MFCs were inoculated with a mixed bacterial culture from the anode of a single chamber MFC, which was originally inoculated with domestic wastewater. Extracellular polymeric substances (EPS) were extracted from the anode biofilm following the procedures reported previously. The EPS in an anode biofilm capable of producing high current density was extracted and their composition was analyzed. Of the total 0.4 mg EPS extracted from the 28 cm² anode biofilm, 20.5 mg (51.2%) was humic substance, 6.5 mg (16.2%) was protein, 5.0 mg (12.6%) was carbohydrate, and 8.0 mg (20%) was unidentified substances, possibly uronic acid, DNA, lipids and/or phenols. The distribution of bacterial cells and polysaccharides in EPS was further analyzed using confocal laser scanning microscope (CLSM). The EPS and the bacteria were found to coexist or overlap in many regions of the electrode surface. CLSM results show various monosaccharides, amino sugar derivatives together with nucleic acids around carbon fibers, and preliminary results suggest that a high concentration of EPS is involved in the electricity-generating biofilm formation.

Cerium oxide nanoparticles in the environment: The electroanalytical techniques face the problem

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The properties of the nanoparticles have encouraged their production and widespread use in our daily life for the last years. Little concern was noticed about the increasing amount of engineered nanoparticles that are released to the environment once the device or product that contains them is of no use anymore [1]. These nanoparticles evolve and interact with the natural media as well as with the living beings that are present in the ecosystem, causing some effects that have to be studied. To fulfil this objective it is necessary to gather as much information as you can about the characteristics of the nanoparticles, like shape, size and concentration in the first place. Several techniques can provide these data but electroanalytical techniques exhibit unique characteristics to approach this problem and offer good answers in simple and fast way.

The authors propose the use of Voltammetry of Immobilized Particles (VIP)[2,3] for the detection and quantitation of nanoparticles even at very low concentration, cerium oxide nanoparticles (CeNP) in this case. This technique can provide relevant data about the oxidation state of the metal in the nanoparticle which affects its reactivity and toxicity. The authors are using it for the characterization of CeNP in solids coming from exhaust pipes of gasoil fuelled motors and water of human consumption where the detection by TEM is difficult due to their tiny diameter, sometimes 2 nm, and because they are scarce in number. The possibility of using VIP for diameter determination is also studied. Particle collision has been another approach that offer clear advantages by comparison to other techniques that offer information about diameter distribution of nanoparticles suspended in an adequate supporting electrolyte [3,4].

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Amperometric Affinity Sensors for the Multiplexed Detection and Determination of Antibiotics Residues in Milk

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Tetracyclines, sulfonamides and β -lactams are among the most common groups of antibiotics employed to prevent and treat animal diseases in veterinary medicine [1, 2]. Milk and dairy products industries are among the sectors most affected by the presence of antibiotic residues, causing a serious threat to human and animal health and also important economic losses in industrial processes [3].

In this work two amperometric biosensing designs for the multiplexed detection and determination of antibiotics residues in milk, involving competitive affinity reactions between the targets and HRP-labelled analogs for the binding sites of specific immobilized bioreceptors, are reported. An amperometric affinity-magnetosensor for the multiplexed detection of cephalosporin, sulfonamide and tetracycline antibiotics residues in milk was developed using disposable electrodes, and a mixture of three specifically targeted magnetic beads sets (**Figure 1a**). Moreover, an integrated amperometric immunosensor for the simultaneous determination of sulfonamide and tetracycline antibiotics, at the low ppb concentration level was developed by immobilization of specific capture antibodies on a novel scaffold constructed by covalent binding of Protein G to pre-grafted, EDC/Sulfo-NHS activated 4-aminobenzoic acid films on disposable dual screen-printed carbon electrodes (**Figure 1b**) [4].

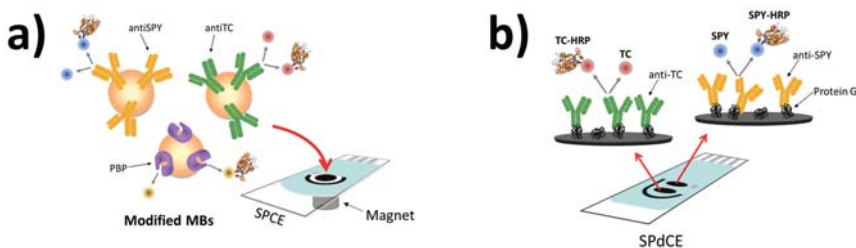


Figure 1.- Schematic displays of the magnetobiosensor (a) and integrated immunosensor (b) for multiplexed determination of antibiotics residues in milk.

The great exhibited performance, together with the use of disposable mass-produced sensors, makes the developed affinity-sensing platforms interesting, useful and affordable alternatives to classical assays for the detection and determination of antibiotics in milk.

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Array of peptide modified electrodes for the simultaneous determination of Pb(II), Cd(II) and Zn(II)

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The use of peptide modified electrodes for the detection and quantification of metal ions in natural samples is an area of major concern [1]. The ability of peptides to bind these metal ions is a consequence of the existence of a great number of potential donor atoms through both the peptide backbone and amino acid side chains. The complexation of heavy metals by thiol rich peptides such as glutathione (γ -Glu-Cys-Gly, denoted usually as GSH), its fragments Cys-Gly and γ -Glu-Cys, or oligomers named phytochelatins ($(\gamma$ -Glu-Cys)_n-Gly, denoted usually as PC_n), and related molecules has been extensively studied using electroanalytical techniques on mercury and bismuth electrodes in combination with multivariate analysis [2]. These works demonstrate that complex formation with the thiol groups from the Cys links plays a crucial role in natural metal binding, as well as for heavy metal detoxification and for phytoremediation.

An essential aspect in the design of peptide modified electrodes is the molecule immobilization procedure. In this sense, the peptide immobilization on aryl diazonium salt monolayers anchored on the electrode surface has demonstrated to be a good strategy that can overcome the major limitations of thiol self-assembled monolayers such as the narrow potential range for metal ion detection. Peptide modified electrodes can be used for metal determination as a single-electrode sensor or in combination with others forming a multi-sensor array, in which each electrode in the array is modified with different compounds in search for a multivariate response. Thus, this work presents the use of three peptide modified sensors in which GSH, Cys-Gly and γ -Glu-Cys were immobilized on aryl diazonium salt monolayers anchored to the surface of graphite-epoxy composite electrodes (GEC) for the simultaneous determination of ppb levels of Cd(II), Pb(II) and Zn(II). The selection of these three peptides is motivated by the fact that previous complexation studies demonstrated that Cys-Gly and γ -Glu-Cys fragments, as well as the pH of the medium, play a particular and important role in each metal binding since the presence of different binding sites in GSH greatly increases the number of possible structures of the complexes. In this respect, the study compares the information about the simultaneous determination of Cd(II), Pb(II) and Zn(II) provided by one peptide modified sensor at both single and multi pH values (pH 6.8, 7.5 and 8.2) with that supplied by the three-sensor array at multi pH values, being the first attempt of using quadrilinear data (sample x sensor x voltage x pH). An artificial neural network model was proposed as a tool to maximize the information obtained from the voltammetric data sets using GSH – GEC, γ -Glu-Cys – GEC and Cys-Gly – GEC sensors that a priori are difficult to interpret [3].

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Electrochemical Aptasensing

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A highly competitive and hot topic in developing biosensors is the use of aptamers, also known as ‘chemical antibodies’, as bio-recognition compound. Aptamers (single strand (ss)DNA or RNA) are synthetic oligonucleic acid sequences which can bind to their targets with high affinity and specificity due to their flexibility. In addition, they are stable and can be employed in extreme conditions. Moreover, these oligonucleic acids can be easily modified by attachment of functional groups without affecting their affinity. Electrochemical sensors with immobilized aptamers as sensing elements are called electrochemical aptasensors. The high selectivity of these sensors is a result of the unique properties of aptamers.

Many strategies are suggested for the immobilization of aptamers on transducers surface but they are mostly restricted by covalent attachment or chemisorption. Despite the fact that aptamers are chemically more stable compared to proteins, they have to be protected from nucleases. For this aim, entrapment in a protective matrix is suggested to overcome this problem. Selecting an appropriate host matrix for aptamers is one of the main challenges for the immobilization of aptamers in order to improve the analytical characteristics of the aptasensors.

In this work, different immobilization strategies will be discussed aiming the development of aptasensing devices for the detection at low levels of environmentally important molecules such as allergens, antibiotics, PCBs, ...

Carbon-based modified electrodes for *o*-toluidine voltammetric detection

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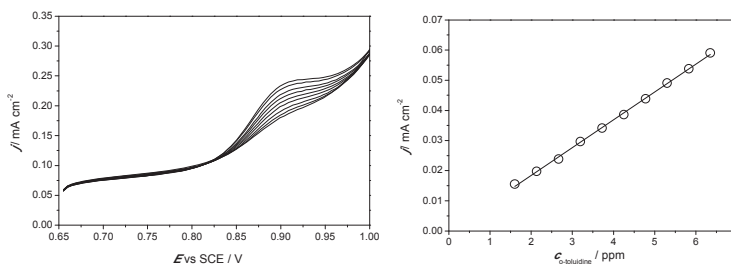
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Different modified electrodes, based on the screen-printing technology (SPE) or prepared by the casting procedure using different carbon materials (carbon nanotubes, graphene, graphite,...) have been prepared and fully characterized by Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS). Also the effect of the use of different supporting materials was studied.

The final devices have been compared with GC electrode, for the development of a new electroanalytical methodology for the detection of *o*-toluidine, an organic carcinogenic synthetic pollutant mainly used as an intermediate in production of azo-dyes, already studied by the research group in a previous work [1].

The developed method is based on voltammetric techniques, which allow to achieve excellent results in terms of large dynamic concentration ranges, high accuracy and precision and low limits of detection and quantification [2]. The use of nanomaterials (in particular carbon nanotubes) enhances the potentialities of the method, improving sensitivity and lowering detection limits [3].

In particular, *o*-toluidine was detected using Linear Sweep Voltammetry in the range 1.5-7 ppm, obtaining a limit of detection of 0.16 ppm and excellent apparent recovery factors (102%) and repeatability, in comparison with carbon based-screen printed electrodes, which presented problems of fouling, probably due to polymerization products.



This new method was used for the determination of *o*-toluidine during its photodegradation mediated by ZnO photocatalyst, showing better performances than C-SPE and comparable with HPLC.

Moreover, the methodology was also employed to monitor *o*-toluidine absorption by cyclodextrine nanosponges based on hydrogel polyamidoamines (PAA), allowing to discriminate among various types of resins and to obtain absorption kinetic parameters.

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Signal amplification by pH-value induced modulation of enzyme activity within a redox polymer film

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Redox enzymes are widely investigated for the electrochemical detection of their substrates, both by mediated and direct electro-communication between enzyme and electrode. The control of the bioelectrocatalytic process by an external stimulus extends their application to follow non-redox reactions in bioelectronic devices. Here we will present a self-powered electrochemical system for signal amplification via activation of a redox-active enzyme. This will be based on the periplasmic aldehyde oxidoreductase from *Escherichia coli* PaoABC [1] in combination with a hydrolase.

The optimum pH for aldehyde electrocatalytic oxidation by PaoABC can be changed from pH 4 to 9 upon coupling with different electron mediating compounds [2,3]. Here, PaoABC is integrated within an Os-complex modified redox polymer and immobilized on an electrode surface. The electrocatalytic current generation in presence of the PaoABC substrate (vanillin) is activated and deactivated by modulation of the pH-value in the electrolyte solution and occurs at the formal potential of the polymer-bound Os complex. Increase of the PaoABC activity, i.e. of the final current output, is shown if the electrolyte solution is acidified.

If this PaoABC containing bioanode is connected to a biocathode based on bilirubin oxidase in direct electron-transfer regime with a carbon nanotube modified electrode surface only a negligible current was flowing. However, upon acidification of the bioanode solution a substantial increase in the biofuel cell current could be detected. When a second enzyme, which is evoking a pH change during turnover, was integrated within the same polymer film as the PaoABC, the local pH could be altered by the addition of the substrate of the second enzyme. For example employing an esterase which catalyzes the methyl propionate hydrolysis, can trigger the PaoABC activity and subsequently a current flow in the biofuel cell. The fuel cell configuration opens the possibility for integration of the signal amplification concept in self-powered sensors.

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Amperometric monitoring of drug penetration through skin

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Transdermal drug delivery represents an attractive alternative to oral delivery especially when poor drug absorption or enzymatic degradation in the gastrointestinal tract or liver is a problem. The greatest challenge is to develop transdermal delivery systems which would enable a large variety of drugs to be administrated by this route.

Stratum corneum (SC), the outermost thin layer of the skin, represents the major resistance to transdermal drug delivery. Penetration of compounds through SC or whole skin membranes is often studied by exploiting flow through diffusion cells or Franz cells. However, the sensitivity of the method is in the range of $\mu\text{g}/\text{cm}^2$ and rapid changes of penetration are difficult to monitor.

In this work we present a methodology where an excised skin membrane is placed directly on an electrode and penetration of different compounds are monitored amperometrically. The proposed method of amperometric monitoring was used to study penetration of different small-molecular weight compounds and to assess the effect of some penetration enhancers. The figure below shows an amperometric response due to the penetration of quercetin ($\log K_{\text{ow}} = 1.82$, $\text{pK}_\text{a} = 7.8$), an example of antioxidant compound. The diffusion coefficient of quercetin in SC was estimated by fitting the amperometric response to a mathematical model. The proposed electrochemical technique is currently evaluated to monitor cutaneous fluxes of hydrophilic and hydrophobic compounds through skin membranes. Penetration of active compounds from topical formulations is also under investigations.

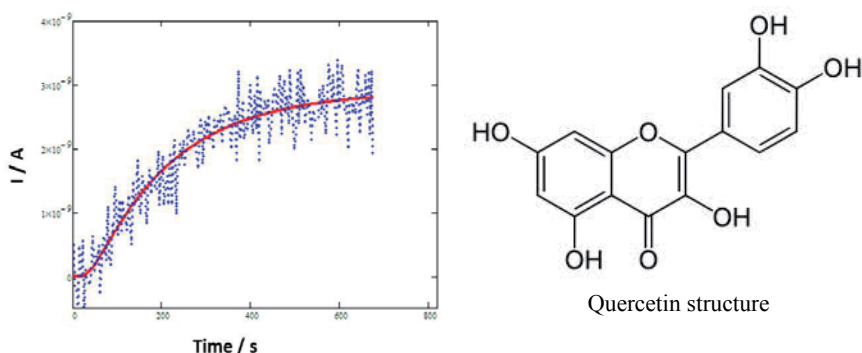


Figure. Amperometric response due to penetration of quercetin through stratum corneum placed directly on the sensor. The measurement was conducted in citrate buffer ($\text{pH} = 4.5$) with 30% ethanol at a platinum electrode modified with carbon nanoparticles.

Photo-electrochemical communication between cyanobacteria and osmium redox polymer modified electrodes

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Most of the energy for all forms of life in the world originates from sun, the ultimate source of energy. Photosynthesis is the process by which green plant, algae and some kind of bacteria convert sunlight into chemical energy with a quantum yield of about 100% [1]. Cyanobacteria, also called the blue green algae account for 20-30% of global primary photosynthetic activity. The electrogenic conduit of cyanobacteria might be exploited to develop light sensitive devices that can convert solar energy into electricity [2]. Recently *Rhodobacter capsulatus* [3] the metabolically versatile purple bacteria has been communicated with osmium redox polymer modified graphite electrode.

In this communication photo-electrochemical study has been investigated with a cyanobacterial species e.g. *Leptolyngbya* sp. CYN82 (collected from the Cawthron Institute Culture Collection of Microalgae, CICCM) with electrode by flexible Os^{+2/+3} functionalities. Photosynthetic constituents in cyanobacteria were excited with a visible light and then the subsequent electron transfer from them to the electrode surface has been documented by cyclic voltammetry and chronoamperometric measurements. A noticeable amount of photocurrent was observed while cyanobacteria embedded on the osmium polymer matrix in the presence of light under anoxic condition.

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Real-time multiparameter monitoring of cellular dynamics - an automated microfluidic electrochemical analysis platform

A. Heiskanen, K. Zór, C. Claudia, M. Vergani, E. Landini, M. Carminati, M. Dimaki, W.E. Svendsen, G. Ferrari, U. Wollenberger, R. Raiteri, M. Dufva, M. Sampietro and J. Ennéus*

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Since the conceptual emergence of microfluidics, many devices have demonstrated capabilities in diverse application areas. During the recent years the most flourishing application area has been cell manipulation and analysis. Although most devices are based on the concept of a small microfluidic chip operated by bulky external instrumentation, a new trend is modularity that encompasses all the necessary automated operations in a small footprint device [1]. In an analogous way as cell biological application are predominantly based on optical/fluorescence based detection, microfluidic devices for cell biology are implementations of optical detection. However, many relevant parameters are detectable using electrochemical techniques. Moreover, electrochemical detection can strongly contribute to automation and portability in device design.

Here, we present a new microfluidic concept that encompasses modularity in both microfluidic and electrochemical meaning. A compact motherboard, which a further development of our previous work for cellular redox assays [2], houses all fluidic operations, temperature control, and gas control needed for automated cellular assays without an incubator (Fig. 1a). Through an optimized integration process microelectrode chips can be easily combined with different microfluidic chip designs. Moreover, the electrochemical experiments can be performed using a previously developed miniaturized 24-channel potentiostat [3], which is custom-made to fit on top of the modular microfluidic motherboard. The system is designed for on-line electrode modification followed by diverse cell-based investigations, and its capabilities have been demonstrated in, e.g., repeated dopamine exocytosis monitoring from cell populations (Fig. 1b), impedance based monitoring of toxic effect of chemotherapeutic substances (Fig. 1c), wound healing, and mediated amperometric monitoring of cellular redox environment.

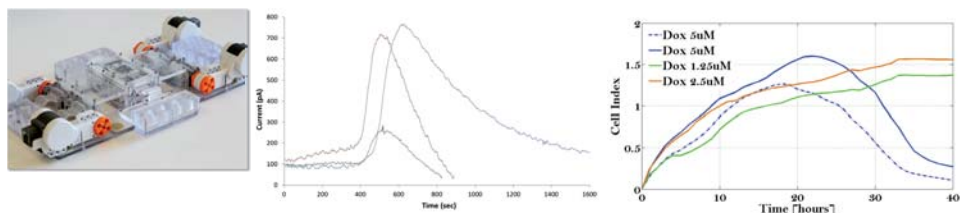


Figure 1. a) Modular microfluidic platform for electrochemical cell-based investigations, such as b) repeated monitoring of dopamine exocytosis from the same cell population, and c) impedance based monitoring of toxic effects of cancer drugs.

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Operation of the in-situ prepared bismuth film electrode in more acidic medium

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Since its introduction in 2000 (1), bismuth electrodes have gained a wide acceptance in numerous electrochemical laboratories worldwide for measuring (trace) metal ions and selected electroactive organic compounds. They can be used in various configurations spanning from bismuth film electrodes prepared in-situ and ex-situ on different carbon-based substrates, e.g. glassy carbon, carbon fibre, graphite etc., gold, platinum, to bulk bismuth electrode, bismuth powder and bismuth-salts modified carbon paste electrodes. Besides a non-toxic character, bismuth electrodes exhibit similar electroanalytical performance to their mercury counterparts, and in certain cases even surpass them, particularly considering insensitivity towards dissolved oxygen. In combination with the in-situ prepared bismuth film electrode (BiFE), several (heavy) metal ions can be readily measured using anodic stripping voltammetry (or stripping chronopotentiometry), usually in acetate buffer solution with pH value adjusted between 4 to 5. However, in more acidic solutions, e.g. in 0.01 M hydrochloric acid with pH 2, its electroanalytical performance becomes hindered, particularly in the case of measuring Zn(II), due to commencement of hydrogen evolution reaction.

In this work a successful anodic stripping voltammetric application of the in-situ prepared BiFE in more acidic medium (pH 2) is demonstrated. The beneficial effect of potassium sodium tartrate was exploited enabling measurement of low concentration levels of Zn(II), also in the presence of Cd(II) and Pb(II), due to induced wider applicable cathodic potential window of BiFE. Several key operational parameters were studied and optimised, and finally, the proposed protocol was tested for measuring Zn(II) in a real sample of tap water.

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Electrochemical Real Time PCR Using Peptide Nucleic Acid (PNA) as Sequence Specific Intercalator

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Sensitive and sequence specific DNA detection is of great importance in many applications. Electrochemical DNA detection has been developed to overcome the disadvantages of bulky and expensive instrument and sophisticated operation in fluorescence-based PCR. In this study, we present an electrochemistry-based real time PCR using peptide nucleic acid (PNA) as sequence-specific intercalator based on an immobilization-free electrochemical detection platform established by our group [1]. As shown in Figure 1, before PCR, the ferrocene-tagged PNA (Fc-PNA) probe is repelled from the electrode due to the electrostatic repulsion between the negative DNA backbone and the negative ITO electrode surface. During the PCR process, a lot of double stranded PCR amplicons are generated. PNA will hybridize with the complementary region on the template and form triplex structures [2]. With increasing PCR cycles, more PNAs will intercalate into the double helix, less Fc-PNAs are accessible to the electrode surface, resulting in decreased electrochemical signal. The working mechanism has been experimentally proved, shown in Figure 2 and Figure 3. The intercalating behavior has shown good specificity by comparing the signal reduction rate between using random DNA (no complementary region to PNA) and target DNA as template in Figure 4. Furthermore, the quantitative analysis of initial target concentration will be studied. The exact intercalating mechanism and the feasibility to distinguish SNP will also be investigated. This electrochemical detection system has high potential for point-of-care applications.

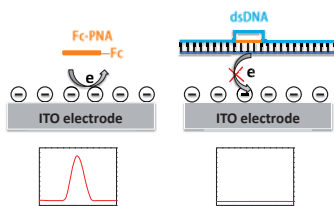


Figure 1. A schematic showing the working principle of the cTag-based ERE-PCR

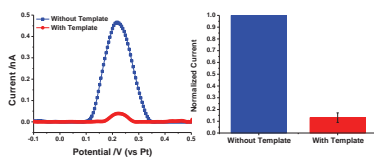


Figure 2. Differential pulse voltammetric (DPV) scans with template or without template.

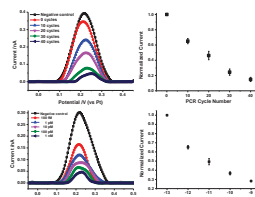


Figure 3. DPV scans after different cycles of PCR and for a series of target concentrations

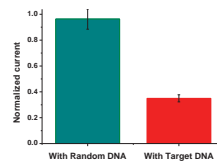


Figure 4. Specificity of PNA intercalating into double helix

Funding support from the Research Grants Council of the Hong Kong SAR Government (Project No. 601212) and the Hong Kong PhD Fellowship Scheme are acknowledged.

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Magnetic beads based electrochemical multiplexed immunosensor for simultaneous determination of coexistent mycotoxins in foods

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Mycotoxin analysis is a highly concern area included in food safety, since they are potent toxins causing a wide range of actions on animals and humans health at low concentrations. Considered as secondary metabolites produced by filamentous fungi, mainly from the *Aspergillus*, *Penicillium* and *Fusarium* genus, present a large amount of toxic effects as carcinogenic, teratogenic, nephrotoxic, hepatotoxic and other important diseases in animals and humans. Due to these adverse properties and its appearance as contaminant in various agroalimentary products such as cereals, cereals-based foods and beverages, sensitive and specific methods for fast, reliable and low cost detection are compulsory. As important analytical tool for mycotoxins monitoring, electrochemical immunosensors combine the inherent selectivity of the antigen-antibody interaction with the well-known advantages of electrochemical detection as high sensitivity, low cost, or in-situ measurements.

In this communication, a sensitive and reliable electrochemical immunosensor for the multiplexed determination of mycotoxins fumonisin B1 (FB1) and ochratoxin A (OTA), due to their co-occurrence in some agricultural and food products, will be presented. The developed electrochemical immunosensors involves magnetic beads as oriented antibody immobilization support, and disposable array of CSPEs for simple, fast and sensitive FB1 and OTA determinations. The use of a screen-printed electrochemical array and a multipotentiostat for conducting eight-in-parallel measurements, allow a multiplexed determination of both mycotoxins including a simplified calibration protocol.

Optimization of the main parameters affecting the performance of the immunosensors such as concentrations of (bio)reagents and immunoassay conditions have been carried out. Under optimized conditions, remarkable limit of detection (well below of the requirements of the European legislation), a good precision and excellent accuracy (evaluated through the analysis of certified reference materials), have been obtained. Furthermore, using a simplified calibration protocol, solid and liquid food samples were analysed showing the excellent suitability of the proposed immunosensor.

The proposed disposable and portable multiplexed immunosensor could be extended to other relevant mycotoxins becoming as a competitive approach in food safety diagnosis as alternative to other more sophisticated instrumental techniques as HPLC-MS.

Acknowledgements: Financial support from the Spanish Ministry of Economy and Competitiveness CTQ2011-28153 (A.E.) and the AVANSENS program from the Community of Madrid (P2009/PPQ-1642) are acknowledged. D. Adrián Jodra acknowledges the FPI fellowship received from the Spanish Ministry of Economy and Competitiveness.

High-speed potentiometric SECM imaging of radially symmetric targets

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Potentiometric Scanning Electrochemical Microscopy is still severely limited by the slow imaging, compared to other microscopic techniques. That is, major changes in the studied system can occur by the time the imaging of a relevant area is completed. The reason for the low speed is the long response time of the potentiometric cell using micro-electrode as scanning probe. If the scan rate is too high, there is not enough time for the potentiometric cell to reach equilibrium before advancing from one data acquisition point to the next, and the image will be distorted (Fig. 1A). Therefore, in order to obtain high quality images with as little distortion as possible, low scan rate must be used.

This is a problem when a rapidly changing dynamic system is studied, or in time dependent studies, where multiple images must be recorded sequentially with sufficiently high temporal resolution, both requiring high scan rate. Imaging such systems, there is a compromise between image quality and scan rate.

We present an approach to alleviate this compromise, allowing high scan rate and high image quality at the same time. We used optimized scanning algorithms based on a polar coordinate system. Their performance has been compared to that of the traditional raster grid based scanning algorithms; the meander, the comb, and the fast comb. With the new “arc” and “web” algorithms, it is possible to complete the scan faster, and obtain higher quality images. Examples of the images obtained with the meander and the arc scanning algorithms are shown in Fig. 1C and E, respectively.

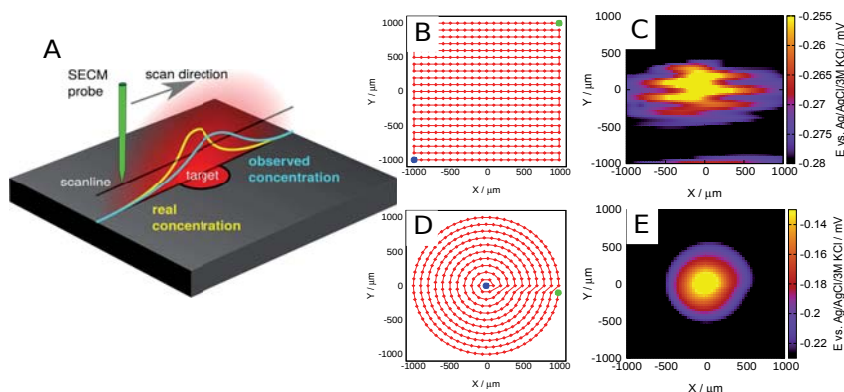


Figure 1 (A) The distortive effect of high scan rate on image quality. (B) The meander scanning algorithm (scan time = 440 s), and the image obtained using this algorithm (C). (D) The new, arc scanning algorithm (scan time = 340 s), and the image obtained using it (E).

Acknowledgements: This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 ‘National Excellence Program’, and TÁMOP-4.2.2.A-11/1/KONV-2012-0065.

Enantioselective recognition at mesoporous chiral metal surfaces

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Chirality is widespread in natural systems and reproducing chiral recognition artificially is a major scientific challenge, especially because of the various potential applications ranging from catalysis to sensing and separation science. In this context, molecular imprinting is a well-known approach for generating materials with enantioselective properties, and it has been successfully employed using polymers. However it is particularly difficult to synthesize chiral metal matrices by this method. We report here for the first time the elaboration of chiral imprinted mesoporous metal, obtained by the electrochemical reduction of platinum salts in the presence of a liquid crystal phase and chiral template molecules. The porous platinum retains a chiral character after removal of the template molecules. A matrix obtained in this way exhibits a large active surface area due to its mesoporosity, and most importantly also shows a very significant discrimination between two enantiomers, when they are studied using such materials as electrodes in Differential Pulse Voltammetry.

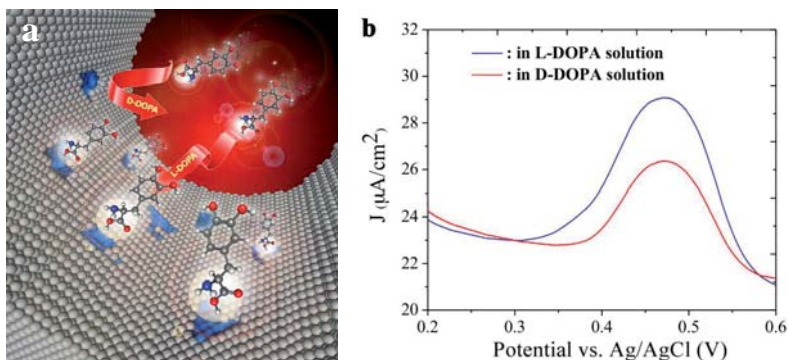


Figure a) Scheme illustrating the inside of a chiral imprinted mesopore **b)** Differential Pulse Voltammetry signal of a mesoporous electrode that has been imprinted with L-DOPA

Reference: C. Wattanakit, Y. Bon Saint Côme, V. Lapeyre, P. A. Bopp, M. Heim, S. Yadnum, S. Nokbin, C. Warakulwit, J. Limtrakul, A. Kuhn, *Nat. Comm.* (2014) 5:3325, DOI: 10.1038/ncomms4325

Electrochemical interaction of antidepressant drug aripiprazole with DNA and DNA damage induced by UV-C radiation and chemical agents

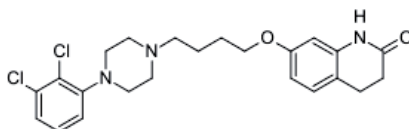
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The investigation of the binding mechanisms of drugs and radiation with DNA helps to understand the structural properties of DNA, the mutation of genes and the origin of some diseases, as well as the action mechanism of drugs and therefore to design new more efficient DNA targeted drugs. DNA damage may cause serious disturbances of the cell life, including mutations or malignant transformations. There are also a few established analytical methods for the identification and quantification of DNA damage products which can be used as molecular markers for genetic toxicity. Aripiprazole (APR) is an atypical antipsychotic agent, which acts through dopamine D2 partial agonism, serotonin 5-HT1A partial agonism and 5-HT2A antagonism. APR is recently approved by the US Food and Drug Administration as the sixth second-generation antipsychotic for the treatment of schizophrenia, schizoaffective disorders, bipolar disorder and adjuvant therapy for major depression.



Structure of Aripiprazole

The electrochemical interaction of calf thymus-DNA with aripiprazole was followed by differential pulse voltammetry. The interaction mechanism and binding constant between calf thymus DNA and aripiprazole was investigated by differential pulse voltammetry and UV-VIS spectrophotometry. The decrease in intensity of the guanine oxidation was used as an indicator for the interaction mechanism and determination of aripiprazole in acetate buffer pH 4.70. A linear dependence of the guanine oxidation signals was observed within the range of $0.2 - 1.12 \times 10^{-6}$ M aripiprazole, with a detection limit of $0.028 \mu\text{g mL}^{-1}$. The proposed electrochemical DNA biosensor method was validated and applied to pharmaceutical dosage form containing aripiprazole. Moreover calf thymus-DNA was damaged by UV-C radiation and some chemicals. The interaction between this damaged calf thymus-DNA and aripiprazole was also determined.

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Selective simultaneous chronoamperometric and piezomicrogravimetric determination of some toxins at electrodes coated with conducting molecularly imprinted polymer films

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Thin films of conducting molecularly imprinted polymers (MIPs) were prepared for determination of several environmental nitroaromatic contaminants (NTs)¹ and nicotine (Nic) with the simultaneous use of chronoamperometry (CA) and piezoelectric microgravimetry (PM). For that, dedicated bis(2,2'-bithienyl)methane derivatives were designed and synthesized to serve as functional monomers. The theoretical DFT calculations, verified by experimental fluorescence or UV-vis spectroscopy titrations, allowed determining stoichiometry and stability constants of the NT and Nic pre-polymerization complexes with the functional monomers formed in solutions. The NT- and Nic-templated MIPs (MIP-NT and MIP-Nic, respectively) films were deposited by potentiodynamic electropolymerization on the Au-film electrodes of quartz crystal resonators (Au-QCRs) from solutions of the pre-polymerization complexes. In this step, NTs and Nic played a role of templates. The imprinting factor ranged from 4.8 to 9.9. Before determinations, the NT and Nic templates were extracted from the respective template-loaded MIP films leaving the imprinted molecular cavities vacant and thus accessible for the NT and Nic analytes. Completeness of the extraction was confirmed by XPS and DPV. The signal of chemical recognition was transduced to the analytical signal of simultaneous changes of the PM resonant frequency and the CA cathodic or anodic current for NTs and Nic, respectively. Concentration detectability of the chemosensors for NTs was in the range of hundreds to tens μM for CA and PM, respectively, with selectivity against common interferences in the range of 2.1 to 4.8. Detectability of the CA and PM chemical sensors for Nic was as low as 40 and 12 μM , respectively. Among them, the CA chemosensor was more selective against the cotinine and myosmine interferences because of the discriminating potential of 1.10 V vs. Ag/AgCl applied. Differences in selectivity to the Nic analyte and interferences were interpreted by the DFT modeling of complexation of Nic and, separately, each of the interferences by a "frozen" MIP-Nic molecular cavity.

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Blood, sweat and tears: power generation using enzymatic fuel cells

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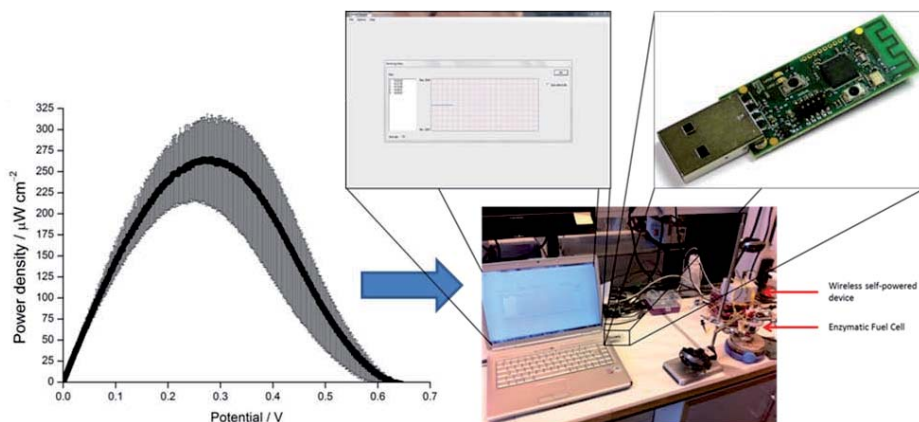
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Immobilization of sugar-oxidizing and oxygen-reducing enzymes on non-catalytic electrode materials, and their combination as anodes and cathodes, offers a route to prototype enzymatic fuels cells (EFC), that may be miniaturized and adapted for portable, or *in-vivo*, power production.

EFC development has focused on direct, unmediated, or mediated, electron transfer between enzyme and electrodes to provide current for sugar oxidation and oxygen reduction. Synthesis of a range of redox mediators and their co-immobilization with the glucose-oxidizing enzymes can offer variation in anode production based on efficiency of electron transfer with enzyme and/or immobilization strategy. Recent reports also highlight advantages to stability and overall current generation by addition of structured supports (such as carbon nanotubes), increasing loading and retention of redox mediators and enzymes [1].

Here we present our most recent results of current and power output from glucose/oxygen enzymatic fuel cells designed to operate in blood, sweat and tear solutions, to provide power to an electronic circuit with pump-charge boosting of voltage to enable powering of electronic devices (such as a potentiostat) and transmitting measured signals to a remote computer (as in figure below).



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Rotating Droplet Electrochemistry: A Simple and Versatile Tool for Monitoring the Kinetics of Molecular and Biomolecular Reactions in Low-Volume Samples

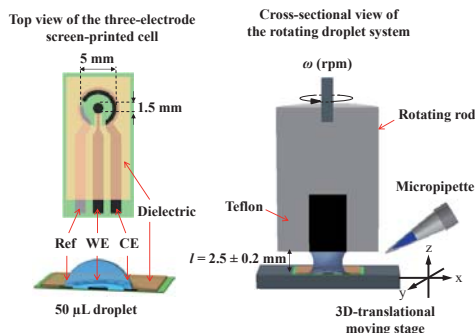
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Monitoring the progress of a chemical or biochemical reaction with an appropriate kinetic technique is essential to determine the rate laws of reactions and to aid in elucidation of reaction mechanisms. This may be accomplished by a wide range of kinetic methods, the most common of which take advantage of changes in optical signals immediately after two or more reactant solutions are rapidly mixed in a chamber. Surprisingly, in spite of the many practical advantages of electrochemical detection methods including high temporal resolution, sensitivity, low cost, and simplicity, only a scarce number of kinetic methods combining rapid mixing of reactants with an electrochemical readout have been so far reported. We propose here a new generic, affordable, simple, versatile, sensitive and easy-to-implement electrochemical kinetic method for monitoring, in real time, the progress of a chemical or biological reaction in a microdrop of a few tens of microliters, with a kinetic time resolution of ca. 1 s.¹ The methodology is based on a fast injection/mixing of a reactant solution (1–10 μL) in a reaction droplet (15–50 μL) rapidly rotated over the surface of a non-moving working electrode (Scheme 1), and on the recording of the ensuing transient faradaic current associated to the transformation of one of the components. Rapid rotation of the droplet was ensured mechanically by a rotating rod brought in contact atop the droplet. This simple set-up makes it possible to mix reactants efficiently and rotate the droplet at a high spin rate, hence generating a well-defined hydrodynamic steady-state convection layer at the underlying stationary electrode. The features afforded by this new kinetic method were investigated for three different reaction schemes: (i) the chemical oxidative deprotection of a boronic ester by H_2O_2 , (ii) a biomolecular binding recognition between a small target and an aptamer,² and (iii) the inhibition of the redox-mediated catalytic cycle of horseradish peroxidase (HRP) by its H_2O_2 substrate.³



Scheme 1

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Electrochemical Impedance Sensor based on Fab'-fragments self-assembled onto hydrophilic gold surfaces

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Antibody Fab'-fragments were immobilised on hydrophilic gold by direct self-assembly. After that they were embedded in a matrix of non-ionic hydrophilic polymers, tris(hydroxymethyl)methylacrylamide, carrying lipoate terminal linking groups. The procedure followed basically the protocol developed by Vikholm-Lundin (1).

Two modified gold electrodes were mounted in a flow cell in a two electrode set-up according to fig 1. Electrochemical impedance spectroscopy was obtained with a 0 V bias between the two electrodes.

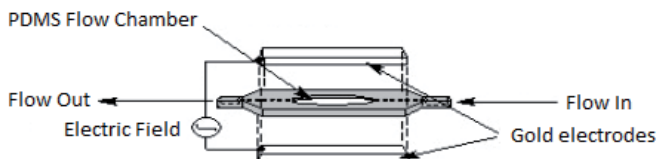


Fig 1: Schematic image of the polydimethylsiloxane (PDMS) fluidic device used for measuring the change in impedance over the frequency range 65kHz to 0,5 Hz between the modified gold surfaces.

The two electrode surfaces within the flow cell were first exposed to Bovine Serum Albumine to cover pinholes in the coated surface to decrease non-specific interactions. Then the surfaces were exposed to IgG in the concentration range, 0,01-100 ppm in 10 mM Phosphate Buffer Saline, pH 7.

The data was analyzed with single value decomposition (2) and by fitting the data to an equivalent circuit.

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Multilayered protein arrangements on electrodes as switchable reaction schemes allowing dual analyte detection

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Biochemical signal pathways are characterized by an effective coupling of individual reaction steps resulting in a rather high specificity in signal transduction. Additionally these complex systems allow a switching between different reaction cascades, depending on defined stimuli, as e.g. the presence of a certain molecule. These efficient biological principles have been adopted for the construction of artificial signal chains.^[1] Protein functionalities can be thus coupled to electrochemical detection schemes, ensuring signal generation in the presence of individual substances.^[2,3]

Interestingly such systems can be built up using proteins as functional building blocks allowing efficient interaction in the immobilised state [4,5]. These multilayered structures are based on cyt *c* self exchange and recently it has been shown that also complex enzyme such as cellobiose dehydrogenase (CDH) can be actively incorporated [6]. Here we report on a novel system which allows an activity-switch between two enzymes co-immobilized in a supramolecular network. This network is formed by embedding the multi-copper enzyme laccase (Lac), the multi-domain enzyme CDH and cyt *c* in an artificial matrix composed of carboxy-modified silica nanoparticles. Immobilisation of all the components is achieved in a layer-by-layer fashion. The build up of the entity is confirmed by quartz crystal microbalance experiments, and investigated by cyclic voltammetry.

Within this layered architecture two enzymes have been connected to the electrode *via* cyt *c*. Since the activity of the enzymes is controlled by the delivery or withdrawal of electrons, the redox state of cyt *c* has been used for switching the activity of the biocatalysts on and off allowing oxygen and lactose detection. Given that the electron transfer throughout the whole layered entity is feasible, Lac and CDH in different distances to the electrode can be addressed. The switchable reaction cascades for Lac and CDH are functioning in a non-separated matrix without disturbing the reaction of the other catalyst.

The approach shows the potential of protein based arrangements and may open the way for the development of multiplex sensing schemes.

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ELECTROCHEMICAL SENSORS USE IN NUTRIENTS EFFICACY ASSESSEMENT AGAINST LIPO- PEROXIDATION

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The wide use of phytochemicals like flavonoids, flavonoid derivatives, polyphenols, carotenoids and anthocyanins from various plant sources as nutrients or as antioxidants preserving or enhancing the food nutritional quality and foodstuff shelf-life made necessary the development of accurate, versatile and in the same time rapid analytical tools to assess them. Among these polyphenols and carotenoids are the most important ones in terms of protective efficiency against damaging factors generating lipo-peroxidation, such as free radicals, radiation or chemical compounds, and, in the same time, ones of the most challenging to be assessed.

The present work is focusing on presenting the availability and versatility of electrochemical sensors as alternative analytical tools in assessment of secondary metabolites from plants and microalgae cultures both as content and as properties evaluation. The developed designs of such analytical devices and corresponding bio-analytical protocols used will be presented.

Biosensors applications on real samples (*Salvia*, *Basilicum*, *Geranium*, *Haematococcus*) limitations and advantages with respect to of their use in phytochemical active principles fast screening will be discussed, emphasizing the antioxidant properties with respect to relevant oxidative markers. Performances characteristics in terms of limit of detection, dynamic response range, selectivity [1,2] while efficacy as antioxidant compounds will be discussed in term of electrochemically measured antioxidant activity against lipoperoxidation. Measuring principle is based on *in situ* generation of peroxyl radicals and lipo-peroxides [3, 4]. The kinetic parameters were calculated out of the experimental data.

Validation issues will be discussed and exemplified using as model compounds both hydrophilic and lipophilic nutrients and comparing the efficacy against *in vitro* induced lipo-peroxidation. LC-DAD-MS and MALDI-ToF analysis provided the validation of electrochemical sensors/biosensors responses.

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ELECTROCHEMICAL GENOMAGNETIC ASSAY FOR QUANTIFICATION OF NUCLEIC ACID TARGETS USING HELICASE-DEPENDENT ISOTHERMAL AMPLIFICATION

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The huge amount of genomic data now available in this post-genomic age is posing new challenges for analytical chemistry. In order to harvest all the available information, there is a growing demand for sensitive, accurate, and easy-to-use methods for the quantification of specific nucleic acid sequences in such diverse fields as food safety, environmental monitoring, and clinical diagnosing. To achieve the high sensitivity required in these areas most of the developed strategies rely on target amplification methods, of which the best known is the polymerase chain reaction (PCR). This is an enzymatic amplification process which requires the use of thermal cycling, limiting its use to centralized laboratories. In addition, classical end-point PCR results in qualitative data, which is not suitable to solve problems where the knowledge of the copy number is critical.

In response, new technologies that mimic *in vivo* DNA replication at a constant temperature might be suitable for developing portable systems that can be used in decentralized analysis such as point-of-care testing. One of the simplest approaches is the so-called helicase-dependent amplification (HDA), which uses a DNA helicase, an enzyme that can unwind the double-stranded DNA under isothermal conditions. Electrochemical genosensors and genoassays represent an attractive option to detect the obtained amplification products in such a way that integrated electrochemical devices that allow rapid, sensitive, and cost-effective detection of specific DNA sequences can be developed.

We here present a sensing platform for DNA quantification which combines HDA amplification and electrochemical detection for the diagnosis of *Mycobacterium tuberculosis* selected as a model system for the development of a general purpose pathogen assay. An HDA assay targeting a short sequence of the IS6110 insertion element, exclusively found within the member of *Mycobacterium tuberculosis* complex, was established. This amplification process gives rise to 84-mer biotinylated amplicons, which are captured on the surface of streptavidin-modified magnetic microspheres and hybridized with appropriate signaling DNA-probes. Finally, the beads are entrapped on the surface of a carbon screen-printed electrode by means of a magnetic field and the signaling probe is electrochemically detected. The analytical performance of the developed assay is described and compared to a PCR-coupled electrochemical detection system. Using this method we have detected the presence of *Mycobacterium tuberculosis* in clinical samples in a few hours.

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A dinuclear thiocarboxylate paddle-wheel Ni(II) complex as unusual precursor of electrochemically generated microstructures for oxidative electrocatalysis

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The search of electrocatalytic materials is the subject of intense study in a wide variety of research areas, such as chemical synthesis, fuel cell catalysis, energy storage and electrochemical sensors. Among many possible electrocatalytic materials, nickel hydroxide has received increasing attention. Although different routes have been described for the formation of nickel hydroxide on surfaces, these methods typically result in thin film structures^{1,2}. The direct *in situ* formation of nickel based structures on electrodes that changes to nickel hydroxide in alkaline medium represents a simpler alternative to the routes already employed. We report here the electrochemically generation of microstructures from an unusual precursor: tetrakis(thioacetate)dinickel(II) complex. The dimetal complex (NiNi) exhibits a dinuclear asymmetric paddle-wheel structure depicted in Figure 1A. It can be electrochemically oxidized to form microbars on gold, glassy carbon and high ordered pyrolytic graphite electrodes (Figure 1B). The size and shape of these microstructures can be modulated upon adjusting the concentration solution and the electrodeposition time. In alkaline medium, the resulting modified electrodes showed potent and persistent electrocatalytic activity toward sugar oxidation. Among them, glucose, fructose, maltose and lactose have been assayed. A catalytic rate constant of $5.36 \times 10^5 \text{ s}^{-1}$ was obtained for glucose (Figure 1C). This high value confirms the great catalytic efficiency of the material, which is also observed to alcohol oxidation. In particular, ethanol, methanol, isopropanol, cyclopentanol and cyclohexanol have been successfully assayed. In this case the best catalytic behavior was observed with primary alcohols. Thus, the resulting modified electrodes can be very useful as sensors for the determination of sugars and alcohols.

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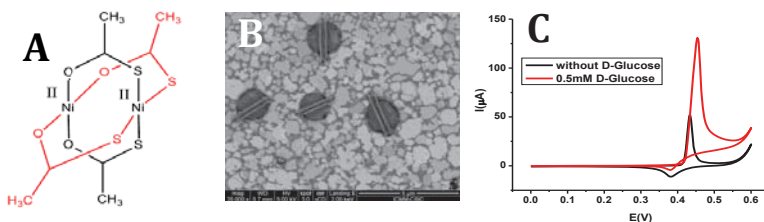


Figure 1. A) Schematic representation of the structure of [Ni₂ (CH₃COS)₄]. B) FE-SEM images of Ni(II) structures electrodeposited on a gold surface. C). Cyclic voltammograms of a GC modified electrode in 0.1 M NaOH solution at 0.010 V s⁻¹ in the absence (black line) or in presence (red line) of 0.5 mM glucose.

The use of nanocarbon paste electrodes for electroanalysis

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Carbon paste electrodes (CPEs) in which a carbon, usually graphite powder, is mixed with a pasting liquid ('binder') to form electrodes were first reported by Adams in 1958^[1]. These are very commonly used for electroanalysis, especially in aqueous solutions, since they offer a reproducible, readily renewable electrode surface. Carbon paste electrodes and their properties have been recently reviewed^[2]. The properties of any carbon paste electrode reflect both the nature (chemical and physical) of the carbon material used, and these of the binder. A huge variety of carbon materials have been used in CPEs ranging from graphites, charcoal, ethyne black, glassy carbon powders, diamond, carbon foams and carbon microspheres. Recently fullerenes and carbon nanotubes have extended the list. Nano-carbon has recently been proposed as useful electrode material which offers similar advantages to multi-walled carbon nanotubes but at close to zero cost^[3]. This material is typically composed of approximately spherical carbon particles, which are often of size ~ 10 nm, commonly aggregated to form clumps of ca. ten or more spheres. The particles are available in a range of sizes.

In the present work we examined the use of nanocarbon in carbon paste electrodes and in particular, make a comparison of the voltammetry behaviour of the resulting electrodes with those made from the more usual graphite powder. Different binders are considered, specifically mineral and paraffin oils. The study of the electrochemical properties of these electrodes towards three different molecules ($\text{Ru}(\text{NH}_3)_6^{3+}$, FcCH_2OH and acetaminophen) was carried out showing excellent analytical performance. The ability to obtain high quality voltammetry from the nanocarbon electrode was demonstrated and simulation of the voltammetry allowed the extraction of electrode kinetic parameters with high precision. The introduction of room temperature ionic liquids into the binder was shown to give no beneficial enhancement, despite their successful use in many CPE systems. Separately the diffusion of FcCH_2OH into the electrode shows that the use of nanocarbon paste electrode can also be exploited electrochemically for the measurement of Gibbs energies of transfer between oil and aqueous phases. We also successfully investigated the electrochemical behaviour and adsorption of acetaminophen on this nanocarbon paste electrode. Finally, the nanocarbon paste electrode was applied to detect total antioxidants in multivitamin drug samples.

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Nanostructured colloidal materials for printable biosensors

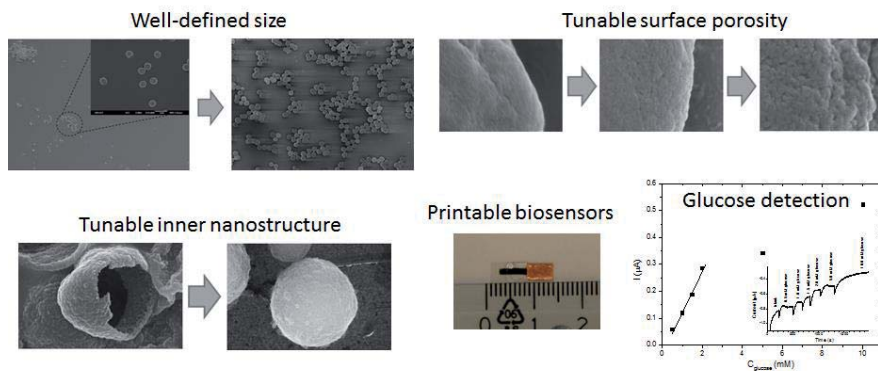
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Abstract

The current trend towards flexible and light-weight portable electronic devices has raised the profile of the emerging field of printed electronics. Printable organic electronic devices provide a high-throughput and cost-effective approach for the fabrication of distributed healthcare devices, which will meet new market needs. Here, we present an innovative modular approach for the design and fabrication of printable biosensors. A “LEGO” style approach comprising various colloidal building blocks with well-defined and tunable nano-scale morphology, electrochemical behaviours and catalytic functions, were fabricated for the development of printable biosensors. The properties of the printable biosensors can be designed simply by varying the composition of the building blocks to fine tune the capacitance, catalytic and affinity functions. This modular approach provides a high flexibility and high throughput method for fabrication of printable biosensors.

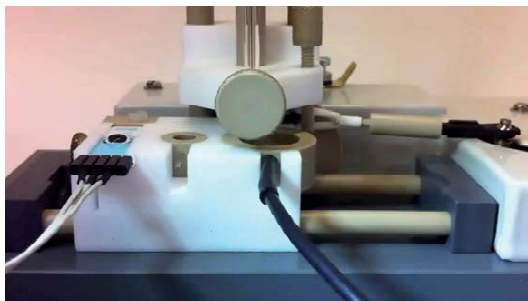


Recent developments of electrochemically assisted injection in combination with capillary electrophoresis – mass spectrometry

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Electrochemically assisted injection (EAI) in conjunction with capillary electrophoresis (CE) was introduced in 2003 as a concept enabling the separation of neutral analytes via electrochemical generation of charged species during hydrodynamic sample injection [1]. The conventional approach for electromigrative separations of neutral analytes is the addition of surfactants forming micelles (micellar electrokinetic capillary chromatography). However, there is a limited compatibility of solutions containing surfactants with electrospray ionization mass spectrometry (ESI-MS). As an alternative EAI-CE-MS can overcome this problem. Our group has developed and characterized several instrumental generations of EAI devices [2-4]. Recently a fully automated EAI-CE-MS system with integrated screen-printed electrodes has been introduced [5]. This system allows a user-friendly operation.



The strength of EAI-CE-MS is particularly seen in cases where charged product species can be formed electrochemically. Bioanalytical studies can be undertaken under near physiological conditions in absence of organic solvents. Detailed studies concerning the simulation of oxidative stress of guanosine will be discussed [6].

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Fabrication and investigation of CNT-based 3D-electrodes for the immobilization of redox proteins

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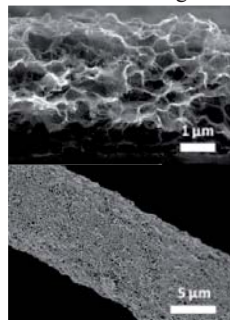
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Carbon-based materials found large application in the domains of electroanalysis and bioelectrochemistry. Meso- and macroporous carbonaceous electrodes already have such attractive characteristics as great porosity, high electronic conductivity and mechanically-stable structure [1]. These features can be even enhanced by combination with carbon nanosized structures which can bring additional catalytic properties for such hybrid materials. This particularly concerns carbon nanotubes that exhibit large surface area and have various active sites on their ends and wall defects. Moreover, they can be modified in order to introduce new functions [2]. The possession of such properties and high pores volume make structured carbon materials attractive for the immobilization of different redox proteins for application in bioelectrocatalysis. Electronic communication between the electrode surface and the redox proteins can then be achieved either by direct or mediated electron transfer reactions. In both cases the large surface developed by porous materials is advantageous. Several applications of such bioelectrodes can be envisaged including highly-sensitive biosensors, biofuel cells and bioreactors.

Here we report different approaches for the fabrication of 3D-structured electrodes based on carbon nanotubes including electrophoretic deposition [3] and layer-by-layer techniques [4]. Prepared therefore electrodes were used as matrix for the enzymes immobilization. The high surface area of carbon nanotubes provides numerous active sites for the biomolecules while 3D-structure of the electrode allows unobstructed diffusion of the reagents.

Prepared by electrophoretic deposition macroporous CNT-assemblies were used for the co-immobilization of sorbitol dehydrogenase and NAD⁺ in the silica film [3]. Such bioelectrode was able to operate in the absence of the cofactor in solution and demonstrated higher sensitivity of sorbitol detection compared with non-porous CNT-electrodes of the same geometric area.

The attention is also given to the application of CNT-decorated electrodes for enzymatic bioreactor. Carbon felt properly modified with CNTs allows immobilization of large amount of enzyme and ensures high transport rate of the reactants. In addition, a sol-gel based silica film contributed to the improvement of the immobilized enzyme stability in the bioelectrochemical reactor allowing high conversion rate of the reactant.



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Colloidal silver-decorated carbon particles in ionic liquid carbon paste electrode for improved determination of nitrite

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Nitrite is used extensively in the food industry to prevent bacterial growth and oxidation/degradation. It is, however, toxic to humans and was found to be a precursor in the formation of nitrosamines and aromatic carbocations, which are known carcinogens. Accurate and direct methods together with reliable sensors for measuring trace nitrite are therefore highly desirable.

A novel carbon paste material was designed for convenient determination of nitrite. Paraffin and silicone oil, commonly used in carbon paste electrodes (CPEs) as binders, were replaced by a room temperature ionic liquid (RTIL), which enhances the conductivity of the CPE material and offers interaction with nitrite by interfacial anion exchange. Trihexyltetradecylphosphonium chloride hydrophobic ionic liquid was used, having a dual effect on the performance of novel electrode, i.e. increased signal-to-noise ratio and possible chloride/nitrite ion exchange with the measurement solution, both of which enhanced the electrode's sensitivity towards nitrite. To further improve the sensor's performance, colloidal silver was grown on the carbon particles using the citrate method at an elevated temperature. Colloidal silver-decorated carbon was shown to have an electrocatalytic effect on nitrite oxidation, which can be observed as a shift of the peak potential and increased signal. Different silver to carbon ratios were tested, with 20 % Ag revealing the most advantageous voltammetric operation. Several key operational parameters for measuring nitrite ions were investigated and optimised. It was found, that the most favourable measuring conditions were in an aqueous solution containing 0.1 M NaCl together with 0.05 M H_2SO_4 using square-wave voltammetric (SWV) mode. Optimization of the SWV scan revealed the most reproducible results together with the highest current signals when the starting potential was set to -0.5 V. The LOD of the novel sensor/method calculated via the 3σ criterium was $10 \mu\text{mol L}^{-1}$; the nitrite oxidation signal was detected at +0.9 V and was highly repeatable with RSD of 0.8% exhibiting no memory effect.

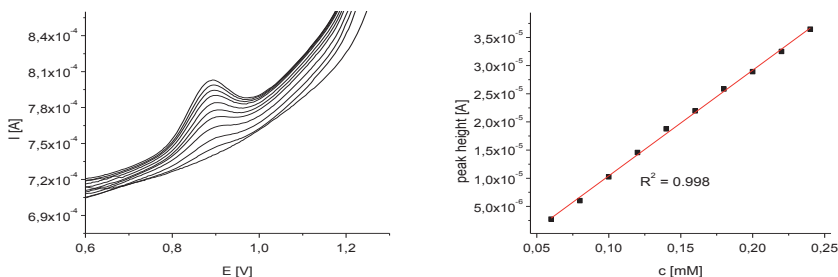


Figure: SWV for 0.06 – 0.24 mM NaNO_2 in $\text{NaCl}/\text{H}_2\text{SO}_4$ and the calibration curve with $R^2 = 0.998$.

Pyrolyzed photoresist carbon electrodes as a new tool for electroanalysis

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The preparation of a novel kind of carbon electrodes obtained by photolithographic micro-fabrication and pyrolysis of an epoxy-based photoresist named SU-8 [1,2] is studied and optimized. SU-8 derived carbon tends to be glassy in nature; however, based on the fabrication and pyrolysis strategies one can obtain a range of electrical, electrochemical and thermal properties related to the tuning of the graphitic content of the obtained carbon. To this aim, the electrochemical behavior of Pyrolyzed Photoresist Carbon Electrodes (PPCEs) is examined as a function of the pyrolysis time and SU-8 film thickness. The results of the electrical, spectroscopic and diffractometric characterization of the PPCEs are reported and discussed with reference to the observed voltammetric performances.

In this work we present the application of this new electrode material to the determination of heavy metal ions. First, bismuth-modified PPCEs (Bi-PPCEs) are used in the adsorptive cathodic stripping voltammetry (AdCSV) of Ni(II) using dimethylglyoxime (DMG) as complexing agent, and a DL of 20 ng L⁻¹ is obtained. In the second application, Bi-PPCEs are used in AdCSV for the speciation of inorganic Cr using pyrocatechol violet as selective ligand for Cr(VI). The analysis of total inorganic Cr species is carried out after oxidation of inorganic Cr to Cr(VI) by irradiating with UV light an oxygen saturated solution of the analyte.

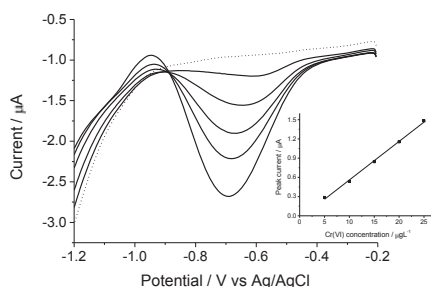


Figure 1. AdSWVs of the ex-situ prepared Bi-PPCE at increasing concentrations of Cr(VI) in the range of 5 – 25 $\mu\text{g L}^{-1}$, in the presence of 5 $\mu\text{g L}^{-1}$ of Cr(III), in 0.5 $\mu\text{mol L}^{-1}$ pyrocatechol violet, 0.01 M acetate buffer solution (pH = 6.0). Accumulation at -0.2 V for 30 s. Square wave parameters: pulse height 50 mV, frequency 25 Hz, step increment 5 mV.

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Electrochemical Phage-sensor for bedside detection of *Pseudomonas aeruginosa*

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Nosocomial infections outbreaks increased significantly these last decades due to many reasons and mainly due to antibiotic resistance development in hospital settings. Among these antibiotic resistant pathogens *Pseudomonas aeruginosa* in association with some other gram negative pathogens is responsible of 90% of nosocomial infection. Our research is focusing on developing an early diagnostic method for *Pseudomonas aeruginosa* pathogen with the aim of rationalising antibiotic de-escalation and improving patient prognosis and survival.

We will show progress in developing a low cost and efficient electrochemical method able to detect specifically *Pseudomonas aeruginosa* in 3h using screen printed microsystems. It relies on combining phage-host interaction with electrochemical-enzymatic detection with satisfying level of sensitivity and selectivity. Highly specific phages against *Pseudomonas aeruginosa* species with wide lysis spectrum were developed. The assay conditions were optimised with whole cell enzyme-linked immunosorbent assay (ELISA) to detect specifically *Pseudomonas aeruginosa* in the presence of competing microorganisms. Although we showed that the selectivity and specificity required for the detection of our target pathogen were reached in this Phage-ELISA assay, this method was time consuming requiring an overnight incubation of phages and not less than 7h to obtain results. However by using electrochemical measurements we were able to improve detection time and specificity by combining phage capture with sensitive specific metabolite detection through enzymatic cascade amplification. We will show a prototype that can reduce to practice pathogen detection in less than 3 hours and without the need of addition of culture media so that regulatory hurdles can be overcome and for its bedside use.

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Biomimetic Studies of Transporting Processes across the Model and Real Phospholipid Membranes

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Every living animal or plant cell is surrounded by a phospholipid membrane (PLM). Across these membranes all compounds and elements, which are necessary for cell life, have to be transported. However, similarly also many undesirable and for cell health dangerous compounds are transported into the cells, where they can start their negative role. In most cases, the transporting processes of such harmful compounds have remained unelucidated. Therefore, using biomimetic tools, our attention has been devoted on one hand to the formation of model cell membranes and, on the other hand, to revealing and to characterization of these processes across these model PLMs as well as across the real cell membranes. We have utilized electrochemical methods (electrochemical impedance spectroscopy (EIS), voltammetry, ion selective electrodes) [1, 2] as well as non-electrochemical methods (optical microscopy, AFM, electrospray ionization mass spectrometry (ESI-MS) [1]) for these purposes.

The attention has been paid to the transport of hazardous metals (e.g., Cu, Cd, and Pb) and of their complexes, mostly with low molecular weight organic acids (LMWOAs) which as small organic compounds play an important role in their transport across the PLMs.

Three different ways of preparation of model PLMs, composed of simple phospholipids (e.g., lecithin) have been realized: liposomes, self-assembling PLMs on the agar surface [3], and PLMs in pores of polycarbonate substrate [1, 4-6]. In order to approach the properties of real membranes, cholesterol [7] and some artificial transporters (e.g., calcimycin [2], polypeptides Transportan TP10) [1] were added to lecithin membranes. Furthermore, the artificial PLMs were mixed with real protoplast membranes (e.g., tobacco, potato, barley). Transporting processes were also studied using pure real PLMs, gained from the above mentioned plants.

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Highly Sensitive Electrochemical Analysis of Heavy Metals with Porous Carbon-Bi Nanoparticle Composite Electrodes

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Heavy metals are persistent environmental pollutants that can be poisonous even at low concentrations. The monitoring of these pollutants in water is being increasingly regulated worldwide. There is, therefore, an increasing interest in the development of analytical tools that allow simple, cost-effective and rapid on-site analysis in contrast to standard methods like mass spectroscopy. In this regard, electroanalytical sensing of trace-level heavy metals using stripping voltammetric approaches is considered an excellent methodology thanks to its low-cost and high sensitivity^[1]. However, composition and microstructure of the working electrode material are critical parameters that directly affect the sensor performance. Bimetallic nanoparticles (Bi NPs) carbon electrodes are an interesting eco-friendly alternative for the fast and ultrasensitive electrochemical detection of heavy metals^[2-3].

We have developed an one-pot synthesis of conductive porous carbon-Bi NPs composite materials via the pyrolysis of organic resorcinol/formaldehyde gels containing well-dispersed Bi³⁺ ions. These materials were further used to manufacture carbon paste electrodes (CPEs)^[3]. The advantages of using those materials include a high active to geometric conductive area of the electrodes together with the high surface area of the Bi NP for the detection of the heavy metals. Electrochemical analysis of Zn²⁺, Cd²⁺, Pb²⁺ and Ni²⁺ were carried out by square-wave stripping voltammetry (SWSV) in batch. Cu²⁺ was also analyzed using a CPEs fabricated with the porous carbon synthesized by the same approach but without Bi. A well-defined and reproducible stripping response was recorded for all the heavy metals tested. Keeping the overall analysis time below 240s, the CPEs showed linear behavior in a wide range of concentrations: 1-100 µg/L for Pb²⁺, Cd²⁺, Cu²⁺; 1-20 µg/L in the case of Zn²⁺ and 10-150 µg/L for Ni²⁺. The estimated detection limits for Zn²⁺, Cd²⁺, Pb²⁺, Ni²⁺ and Cu²⁺ were 0.42, 0.49, 0.53, 7.84 and 0.65 µg/L, respectively.

Moreover, the nanocomposite electrodes were employed to analyze different types of real water samples, such as river water, acid mine drainages and treated urban water, thus covering a wide spectrum of matrices and heavy metal absolute and relative concentrations. The obtained results were in good agreement (with a margin of error of +/- 10%) with those obtained with inductive coupled plasma mass-spectrometry. These results demonstrated the potential of the porous carbon-Bi nanocomposite-based electrodes for the stripping voltammetric detection of heavy metals.

Finally, it is worth mentioning that the synthesis approach of the porous C-Bi NPs composites is compatible with different fabrication processes of miniaturized electrochemical devices of planar configuration, from thick-film (screen-printed) to thin-film miniaturized electrodes. Initial results about the fabrication and performance of these devices will also be shown.

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The development of an electrochemical cytotoxicity sensor "TOXOR" – Applications in environmental toxin monitoring

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Chemicals from industrial and agricultural sources can have a negative effect on human health and the environment. The REACH directive EC (1907/2006) was introduced by the European Union in June 2007 and is concerned with registration, evaluation, authorisation and restriction of chemical substances that may pose a risk to human health and the environment. This places the responsibility on industry to manage any risks from chemicals and provide safety information for them. Mammalian cell biosensors are valuable tools that can be used to assess the cytotoxicity of toxic chemicals. The "Toxor" electrochemical cytotoxicity sensor presented here (see Fig 1) is a mammalian cell electrochemical biosensor that measures changes in cellular enzyme activity following exposure of cells to toxic chemicals. It is envisaged that this device could be exploited in the screening of industrial and environmental toxins and has the potential for pharma/drug testing applications. The integrated electrochemical/fluidic sensor has the ability to measure the activity of the enzyme acid phosphatase in A549 human lung epithelial cells. Acid phosphatase catalyses the conversion of 2-naphthyl phosphate to 2-naphthol (determined using chronocoulometry) and is indicative of metabolically active cells. Immobilised cells exposed to toxic chemicals such as pentachlorophenol, nickel chloride and potassium dichromate showed a decrease in acid phosphatase activity which was detected electrochemically, allowing IC₅₀ (50% reduction in acid phosphatase activity) values of toxic chemicals to be reliably and conveniently determined.

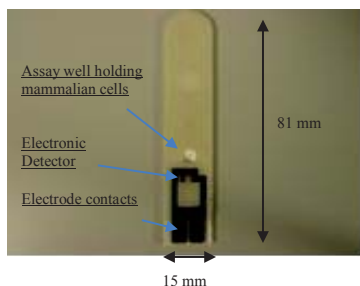


Figure 1 Image of prototype "TOXOR" device with fluidic release system, assay well and electrochemical detector.

Oxygen-terminated Boron-doped Diamond Electrodes in Electroanalysis of Biologically Active Organic Compounds

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Boron-doped diamond (BDD) is since its introduction in 1992 the subject of considerable interest as an electrode material. Its favorable electrochemical properties include low and stable background current over a wide potential range, corrosion and fouling resistance, low sensitivity to dissolved oxygen, and long-term response stability [1, 2]. The electrochemical behavior of BDD electrodes strongly depends on their surface properties such as grain size, crystallographic orientation, surface termination, and sp^2 content at the film grain boundaries. These are influenced by the type and conditions during the preparation using a chemical vapour deposition procedure, boron doping level, and conductive support [3].

The aim of this contribution is to present recent outputs of the BDD-related research of UNESCO Laboratory of Environmental Electrochemistry in the field of electroanalysis of biologically active organic compounds. Both, commercially available and in laboratory-deposited oxygen-terminated BDD electrodes have been used for detection of both, electrochemically reducible and oxidizable organic compounds [1, 2, 4]. The following aspects will be emphasized: (i) Influence of boron-doping level on electrochemical properties of BDD electrodes and their electroanalytical characteristics for batch voltammetric methods including potential window in commonly used buffers, mixed aqueous/organic electrolytes, and determination of model oxidizable and reducible organic compounds (*e.g.*, 2-aminobiphenyl, stigmasterol, nitroquinoline); (ii) Possibilities of utilization of BDD electrodes for detection of organic compounds using adsorptive stripping voltammetry in the presence of surfactants; (iii) Performance of BDD electrodes in liquid flow methods in thin-layer and wall-jet amperometric detection cells for determination of submicromolar concentrations of organic pollutants in environmental matrices.

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Interaction of Biphenyl and Its Derivatives with Model Lipid Membranes

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Many different model lipid membranes have been widely used to study the interaction of membrane active compounds with the biological membrane[1] copycats to access their potential vulnerabilities in the biological environments. Monolayers of phospholipids on Hg surface act as a biosensor for the hydrophobic compounds containing two or more aromatic rings and many others[2, 3]. This system have a toxicity sensing application based on two characteristic phase transitions in response to applied electric fields due to the structural changes in the phospholipid monolayer[4]. Biphenyl and their differently substituted derivatives have been used as dielectric fluids and in the preparation of pesticides, plastics and optical brighteners and are established environmental toxins as well[5]. Electrochemical impedance and fluorescence spectroscopic techniques have been used to study the interaction between biphenyl derivatives and Hg supported phospholipid monolayers and liposomes respectively. Interaction of biphenyls with phospholipid monolayer/bilayer membrane depends on the effect of substituent on the available electron density/polarisability and three dimensional orientations of aromatic rings. Biphenyls with more available electron density are proficient in incorporation and binding to the lipid head groups. On the other hand, penetration into lipid membrane is the experiential process in biphenyls with less available charge density. Biphenyl is a planar structure and ortho substitution causes the distortion in the molecular structure[6] responsible for the least interaction between the ortho substituted biphenyls and phospholipid membrane. Para substituted biphenyls appeared to have strong effect on their binding and penetration into the membrane.

This talk reports on the above findings arising from a comprehensive investigation into the interaction of substituted biphenyls with phospholipid layers under the influence of electric field. Together with parallel investigations using fluorescence spectroscopy, it shows explicitly the binding and penetration of these species into the membrane depending on their chemical structure.

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Electrochemical Direct diagnosis of Cancer and Monitoring of Anti-Cancer Drug Efficacy

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The feasibility of utilizing miniaturized biosensor for cancer diagnosis and treatment efficiency utilizing the electrochemical distinction of cancerous cells activity will be described.

First we demonstrate a multiplexed amperometric biosensor for colon cancer diagnosis based on the difference in the amperometric signal between cancerous and normal epithelia, relying on the observed down-regulation of Alkaline Phosphatase (AP) enzyme secretion by the cancerous cells. Detection of AP activity enabled to distinguish cancer tissue from healthy tissues, according to the expression level of the enzyme. Moreover, we offer the possibility of sampling small volume biopsies rather than cell cultures thus eliminating the need for pretreatment and strenuous preparation steps.

This system was also applied the detection of colon cancer cells response to differentiation therapy. We have design and built a novel electrochemical 'lab on a chip' system that contains an array of nano volume electrochemical cells on a silicon chip. The efficacy of each of the differentiation inducing agents was evaluated through electrochemical detection of the cellular enzymatic activity level. The results demonstrate the ability to evaluate simultaneously multiplex drugs effect on miniature tumor sample (~15 cells) rapidly (5 min) and sensitively, with quantitative correlation between the cancer cell number and the induced current.

In addition, we have tested the effect of several prodrugs on human brain cancer cells (Neuroblastoma U-251) by measuring released formaldehyde in response to treatment with anticancer prodrugs, of butyric acid. The prodrugs were added to U-251 cells situated in the biosensor chamber and the current induced by added prodrugs was monitored. The sensor is rapid, sensitive, selective, inexpensive and disposable, as well as simple to manufacture and operate.

In conclusion, electrochemical biosensors as cancer diagnostic tools present exciting opportunities and may pave the way towards decentralized clinical applications.

Biofuel cells using redox polymer-wired hydrogenases, photosystems or sugar oxidising enzymes

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Redox polymers are frequently used for wiring biological recognition elements such as e.g. enzymes with electrode surfaces. Evidently, for the design of biofuel cells or biobatteries the adaptation of the redox potential of the polymer bound redox species to the formal potential of the prosthetic group in the active site of the enzyme is of high importance. Moreover, the polymer backbone structure has to be modified to allow for high mobility of the polymer-bound redox relays, swelling of the hydrogel etc.

The following aspects will be discussed:

1. Os-complex and phenothiazine-modified redox polymers for wiring of cellobiose dehydrogenase, glucose oxidase and PQQ-dependent glucose dehydrogenase
2. Design and optimization of photobioelectrochemical devices based on photosystem 1 and photosystem 2
3. Viologen-based redox polymers for wiring of Ni-Fe-hydrogenase

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Electrochemical determination of total protein concentration

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Micro- and nanoliter volumes of noninvasively sampled physiological fluids can be analysed by electrochemical methods aiming at simultaneous analysis of several analytes [1]. To minimize the effects of, e.g., sample dilution, it would be of high importance to characterize the sample by some kind of general parameter. Such a parameter could be the total protein concentration in the sample. With this motivation in mind we have investigated the possibility to exploit silver electrochemistry for detection of total protein concentration. The method is based on electrochemical oxidation of silver in chloride containing electrolyte in the presence of protein. The oxidation produces AgCl and an Ag-protein complex. These complexes can be electrochemically reduced giving a measure of the amount of charge which is proportional to the protein concentration. The mechanism has been previously reported [2] but has, however, not been further studied or explored for analytical purposes.

The initial experiments were done by oxidizing and reducing a solid silver electrode in phosphate buffer solution containing 0.1 M KCl in the absence and presence of bovine serum albumin (BSA). In optimizing the electrochemical protein analysis method, we found out that the potential scan rate (2 mV/s), the end positive potential (125 mV vs. Ag/AgCl) and the presence of SDS (2.5 mg/mL) considerably influenced electrochemical determination of BSA. The reproducible regeneration of the surface of solid silver electrode and discrimination of the two reduction peaks at low BSA concentration were identified as two obstacles. Subsequent studies were conducted using gold electrode modified with silver nanoparticles which was found to improve the reproducibility of the method. The analytical performance of this sensor was assessed for the determination of total protein concentration in human saliva samples. At the moment, EQCM-D and electrochemical techniques are combined to deeper understand the process at the electrode surface during Ag⁺ interaction with protein molecules and reduction of the Ag-protein complex.

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Carbon nanochips supported Pt_2Ag_1 and $\text{Pt}_1\text{Ag}_1@\text{Pt}_1$ nanostructured materials for methanol electro-oxidation

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Functionalised carbon nanochips (f-CNC) supported Pt_2Ag_1 and $\text{Pt}_1\text{Ag}_1@\text{Pt}_1$ based nanomaterials were synthesised using modified polyol process. The structural and morphological aspects of the synthesised nanostructured materials were characterised using transmission electron microscopy (TEM/DF-STEM), energy-dispersive X-ray spectrometry (EDX), thermo-gravimetric analysis (TGA/DTG), X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). The TEM analysis (shown in Figure 1) confirmed the successful nanoparticle decoration (average nanoparticle size 2.1 ± 0.08) and upon characterisation, alloy ($\text{Pt}_2\text{Ag}_1/\text{f-CNC}$) and core-shell ($\text{Pt}_1\text{Ag}_1@\text{Pt}_1/\text{f-CNC}$) nanostructures were confirmed. Cyclic voltammetry (CV) was employed for electrochemical studies. The efficient utilisation of both support material and precious Pt for the methanol electro-oxidation are the attractive features of the proposed materials. These nanoscale materials are capable of mass production and have the potential to be exploited in catalysis, electrochemistry and sensing applications.^[1-3] The novel support material (carbon nanochips) combined with the fact that the functional groups on carbon nanochips can be well controlled providing an excellent material for nanoparticle decoration with effective dispersion and utility of Pt.

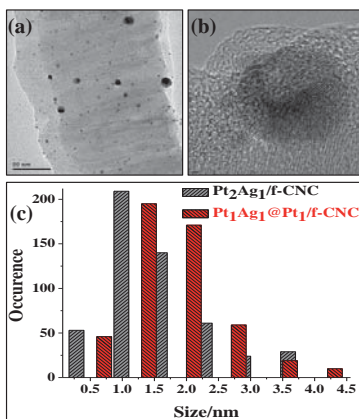


Figure 1. (a) Transmission electron micrographs showing nanoparticle decoration and distribution, (b) high resolution transmission electron micrographs for $\text{Pt}_2\text{Ag}_1/\text{f-CNC}$ and (c) particle size distributions for $\text{Pt}_2\text{Ag}_1/\text{f-CNC}$ and $\text{Pt}_1\text{Ag}_1@\text{Pt}_1/\text{f-CNC}$.

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Electrochemical transformation of some drugs studied using electrochemistry and mass spectrometry

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Electrochemistry combined with mass spectrometry can be used as a powerful tool to elucidate mechanism of redox transformation of different biologically active compounds. Electrochemical cell can serve as a simple model system in which oxidation or reduction reactions, frequently occurring in living organisms, may be simulated within the wide range of experimental conditions. Among others, the approach is usable during the development of new drugs for screening of prospective products of oxidative metabolism (phase I mainly) as well as for electrosynthesis of respective metabolites. From analytical point of view the knowledge of principle and mechanism of reaction proceeding at the working electrode in the electrochemical cell can be used for development of sensitive analytical methods for determination of target electroactive species using various electrochemical techniques including voltammetry, amperometry and coulometry, without or, preferably, after separation.

In the present work electrochemical transformation of an antimuscarinic drug fesoterodine, its main active metabolite 5-hydroxymethyl tolterodine and an antidiabetic drug repaglinide were studied. Anodic behaviour of the three compounds was characterized using cyclic voltammetry at the glassy carbon electrode. Measurements were performed in aqueous-methanolic media of different pH and at different scan rates. Control potential electrolysis of the drugs was performed at the platinum gauze electrode in small volume cell. The electrolyzed samples were analysed using liquid chromatography with electrospray ionization quadrupole time-of-flight mass spectrometry. Electrochemical oxidation pathways of all three drugs were proposed. Identified products were compared to those of enzymatic transformation in cytochrome P450 system.

Acknowledgements

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Electrochemical Imaging of Single Cell Warburg Effect

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Cancer is one of the most leading causes of death in the world; almost 9 million of deaths were counted in 2010. [1]

Most of the tumors are characterized by a switching of cellular metabolism; this set of reactions and processes take place in the mitochondria and use nutrients, such as glucose, as a fuel source. In normal cells and under aerobic conditions glucose is metabolized to adenosine triphosphate (ATP) by oxidative phosphorylation, for meeting the cell's energy demands. However, in cancer cells, much of the glucose is directed away from the mitochondria to create lactate. Lactate production is typically restricted to anaerobic conditions nevertheless cancer cells preferentially channel glucose towards lactate production even when oxygen is plentiful, this process is termed "aerobic glycolysis" or *Warburg Effect*. It was demonstrated that under aerobic conditions, cancer cells metabolize, approximately tenfold, more glucose to produce lactate in a given time than normal cells. [2]

As a consequence, investigation of the metabolic differences (glucose uptake and lactate release) between nonmetastatic and metastatic cells could be a powerful tool in cancer field.

Enzyme-based ultramicroelectrodes (UMEs) in conjunction with scanning electrochemical microscopy (SECM) can be developed as a useful technique for studying cell metabolic fluxes, since it can map chemical activity across the entire surface of a single cell with high spatial resolution and it can record dynamic changes. Enzyme-based sensors offer high selectivity toward a single analyte, based on their structural complementarities, and the opportunity to improve sensitivity, time scale and information content.

Information about the way a cell performs glucose uptake can be acquired using modified UME biosensors with glucose oxidase (GOx) and lactate oxidase (LOx). [3-4] Glucose or lactate are measured indirectly by amperometric oxidation of hydrogen peroxide, that is formed in aqueous environment during the reaction catalyzed by the enzyme entrapped in the proximity of the electrode surface as described by the follow equations:

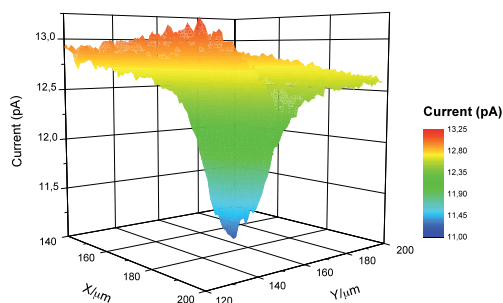
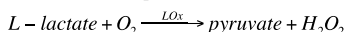
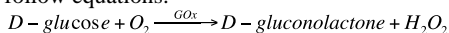


Figure 1. Glucose uptake profile for a single cell using an electropolymerized GOx UME biosensor.

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Electrochemical Detection of Serum Circulating MicroRNA based on Using Methylene Blue Modified Probe DNA: Extending the Application to the Dispersible Electrodes

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Abstract MicroRNAs are an emerging class of diagnostic markers that can signify the presence of disease and be used to predict its course. In addition to their regulatory role in a wide range of metabolic processes, some miRNAs are directly involved in human cancers. For example, levels of microRNA miR-21 have been found to be elevated in more than 10 different types of cancers, compared to their respective healthy controls. Due to their presence in the serum in remarkably stable forms, serum circulating microRNAs are considered as promising novel biomarkers for early cancer detection and improved cancer screening [1, 2].

Recently electrochemical nucleic acid-based biosensor technologies have begun to attract attention for genetic analysis owing to the low background typically observed in clinical samples, and convenience of microelectronics [3-5]. In this research on a method for detection of serum circulating microRNA, we use an electrochemical approach based on using a methylene blue labelled probe DNA as the biorecognition unit. The probe DNA is immobilized on a gold electrode via thiol chemistry, and the other distal end of the probe is labelled with a methylene blue redox probe. Signalling of this sensor originates from the binding-induced changes in the dynamics of the redox-labelled probe DNA, which is dampened by the decrease in the flexibility of the probe-target duplex, leading to a significant reduction in the redox current after hybridization with miR-21. The signal could be recovered to almost $89 \pm 6\%$ of its original value after 30 second brief rinse in deionized water. The sensor also can differentiate between target, single and multiple mismatch base pairs.

To further improve the detection limit and response time of the sensor for point of care applications, we extend the application of this detection strategy to a rapid and ultrasensitive electrochemical approach based on using conductive gold coated magnetic nanoparticles as dispersible electrodes, which serve as the active element in the selective capture and direct electro-analytical quantification of analytes [6, 7]. Modified gold coated magnetic nanoparticles with redox-labelled probe DNA interact with target microRNA-21 as they diffuse through the solution. A magnetic field then brings the particles quickly back to a macroelectrode, where the amount of microRNA-21 quantified electrochemically.

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Elimination procedure in square-wave voltammetry

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Square-wave voltammetry (SWV) is one of the four major voltammetric techniques provided by modern computer-controlled electroanalytical instruments. Applications of square-wave voltammetry include the study of electrode kinetics with regard to the preceding, following, or catalytic homogeneous chemical reactions and determination of some species at trace levels. In this communication we present the application of the elimination procedure to SWV results. Using the different dependence of a linear sweep voltammetric current component (diffusion, charging, kinetic...) on scan rate, EP is capable of conserving or eliminating some current components. The variable parameter for a desired elimination in SWV is square-wave frequency, and even though it is a pulse method which suppresses the influence of the charging current, the elimination procedure increases the sensitivity of SWV by one order. For a totally adsorbed electroactive particle, EP (eliminating simultaneously the kinetic and charging currents) also provides a specific signal in the form of peak-counterpeak and from the electroanalytical point of view this type of signal does not require a baseline correction. Our application approach was verified by means of reduction and oxidation signals of short synthetic oligonucleotides including the presence or absence of a homogeneous chemical reaction preceding the electron transfer. It is shown that SWV in connection with EP is a new powerful electrochemical technique that can be applied in both electrokinetic and quantitative determination of redox couples strongly immobilized on the electrode surface. Generally, treatment of voltammetric data by the elimination procedure gives a new dimension to voltammetric methods.

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Screen-printed graphite microbands for electroanalysis

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Usage of working microelectrodes offers a variety of benefits for electroanalysis. The establishment of convergent diffusion leads to enhanced mass transport which is the most important advantage of microdimensional electrodes and results in an improvement of the analytical performance in comparison with macroelectrodes under planar diffusion. Low capacitive currents, due to the small surface area, contribute to higher signal-to-noise ratios. In order to overcome the problem of low currents detectable at single microelectrodes, multiplication into a microelectrode array is one of the possible approaches, which combines the advantages of enhanced mass transport and high output signals.

The microband is one of the most cost-effective and easily fabricated geometries of microelectrodes. The microband width is a microscopic, critical dimension of the electrode, maintaining convergent diffusion domination, whereas the microband length is macroscopic, which ensures registration of relatively large currents.

Graphite screen-printing on plastic support as a standard technology for scale up production of low cost electrochemical devices combined with simple scissor cutting has been adapted for fabrication of microband arrays. Cutting procedures can easily be adapted for automatic electrode recovery, which might be used in the construction of electrochemical sensors for autonomous environmental monitoring.

Single layer and multilayer microband arrays of different band lengths were produced and characterized with optical and electrochemical methods [1]. The microband width of the electrodes assessed with electrochemical methods was about 5 microns. Electrochemical responses obtained at the microband arrays showed convergent diffusion domination. The developed electrode structures have been used as a versatile platform for formation of model electroanalytical systems. Up to 4 times sensitivity increase has been achieved at the microband arrays for direct oxidation of ascorbic acid. Electrochemical glucose oxidation catalyzed with mediated oxidase immobilized inside a sol-gel membrane on the arrays yielded up to 3 times sensitivity increase in comparison with a macroelectrode.

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High spatial resolution of single cell exocytosis studied with microwell-based ultra-microelectrode arrays

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The distribution of exocytotic activity has been found to be spatially heterogeneous at the surface of a single cell, resulting in hotspots where neurotransmitters are released more frequently (1). This subcellular heterogeneity across a single cell has thus motivated the design of microelectrode arrays (MEAs) capable of resolving the spatial variation of exocytosis across a single cell. However, few papers report the use of thin-film MEAs with individual electrodes smaller than 5 μm (the typical size of the carbon fiber microelectrode used for single cell analysis) for cellular resolution of exocytosis at the basal side of the cell. The development of MEAs with electrodes small enough to allow quantitative measurement of released molecules from exocytotic hot spots distributed on the surface of a single cell is highly important to our understanding of the exocytosis process.

Here, we present the fabrication, characterization, and application of microwell-based MEA devices for high spatial resolution of release at single cells. The microwell-based MEAs consist of up to thirty-six 2- μm -width square ultra-microelectrodes, all inside a 40 $\mu\text{m} \times 40 \mu\text{m}$ SU-8 square microwell. The microwell is used for single cell trapping and single cell culturing on the surface of MEAs. Effective targeting and culturing of single cells in the microwell are achieved by these cell-sized microwell trapping and micropipette picking techniques. Imaging the spatial distribution of exocytosis at the surface of a single PC12 cell has been demonstrated with this system. Figure 1 shows exocytotic signals from 8 independent 2- μm -wide ultra-microelectrodes at a single PC12 cell demonstrating the subcellular heterogeneity in single-cell exocytosis.

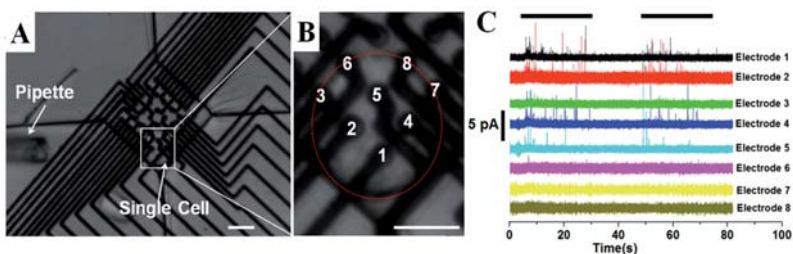


Figure1: A) Micrograph of the setup, showing the microwell-based 36-electrode array partially covered by a single PC12 cell (scale bar: 10 μm); B) expanded view of the electrode array showing a single cell identified in red dotted circle and the labelling of the electrodes (scale bar: 10 μm); C) Representative amperometric traces of exocytotic release from a PC12 cell recorded using an eight-electrode traces obtained for 25-s stimulations of the cell (the stimulations are indicated by the black bars);

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Electrochemical magnetic immunosensors for the simultaneous determination of ochratoxin A, fumonisin B1 and deoxynivalenol

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Mycotoxins are a large and varied group of mold-secondary metabolites produced by fungi that have very toxic effects in human and animals. Filamentous fungi produce thousands of mycotoxins, the more important are aflatoxins, ochratoxins (e.g. ochratoxin A, OTA), and trichotecenes such as fumonisin B1 (FB1) and deoxynivalenol (DON).

Because of their importance and toxicity, the European Union have very restrictive and specific regulations for the occurrence of OTA, FB1 and DON in foods (e.g. in cereals), with the objective of protection of the consumer's health, together to describing procedures for decreasing amounts in food commodities. Official methods for determinations include high-performance liquid chromatography with fluorescence (OTA, FB1) and UV-visible (DON) detections. Immunochemical methods (e.g. ELISA, enzyme-linked immunosorbent analysis) and immunosensors (mainly fluorescent and electrochemical) are increasingly important for the rapid and sensitive control of these.

We have reported individual electrochemical aptasensor and immunosensors for the analytical determination of ochratoxin A (OTA), FB1 and DON [1], which were based on functionalized magnetic beads separations under an external magnetic field. In this communication, we describe a multisensory device combined with a multiplexed potentiostat that conducts eight in-parallel electrochemical measurements, allowing multiple samples, replicates and all the corresponding controls of the three mycotoxins to be measured at the same time. We have previously extracted OTA (*aspergillus*) together with FB1+DON (*fussarium*) from cereal samples using an optimized aqueous-methanol mixture based procedure.

The device allows the multi-mycotoxin determination of the three obtaining sensitivities bellow the allowed limits of the European Legislation (about 2 ng/g for OTA and 200-1750 ng/g for FB1 and DON), and having comparable concentrations with those obtained with official HPLC-FLD and HPLC-UV-visible methods, with commercial ELISAs, and with spectrophotometric magnetic ELISA immunoassays that we have also developed.

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Application of Graphene Based Nanocomposite in Electrochemical Biosensor

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Abstract

The modification of electrodes offers the possibility for electrochemical detection of electroactive and biological molecules. Graphene is a novel material and has attracted considerable attention in electrode modification due to the excellent chemical, electronic and mechanical properties [1]. Moreover, graphene or reduced graphene oxide (RGO) produced by the reduction of graphene oxide possess some characteristic functional groups such as hydroxyl (–OH) and carboxyl (–COOH) groups, which would benefit the preparation of composites [2]. Prussian blue (PB) has shown excellent catalytic effect towards the oxidation-reduction of some low molecular-weight molecules such as oxygen and hydrogen peroxide due to its special three-dimensional network structure which allows the diffusion of the small molecules [3]. Most of the procedures for the deposition of PB are based on electrochemical methods. This research work describes a simple procedure to prepare a novel amperometric biosensor based on reduced graphene oxide – gold nanoparticles – Prussian blue (RGO-AuNPs-PB) composite through spontaneous deposition of PB on RGO-AuNPs surface. The PB component of the resulting composite displayed an excellent stability in neutral electrolyte through the π - π interactions between RGO and the –CN groups together with the excellent affinity between AuNPs and PB. The RGO-AuNPs-PB composite was characterized by scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS). Cyclic voltammetry and amperometric measurements were employed to investigate the electrochemical properties of the modified electrodes. The RGO-AuNPs-PB composite was then utilized to fabricate electrochemical biosensor for the detection of hydrogen peroxide. Experimental results showed that the GC/RGO-AuNPs-PB modified electrode offered good electrocatalytic activity toward the reduction of hydrogen peroxide, indicating the possible synergistic effects of the RGO-AuNPs-PB composite material. The electrode showed high sensitivity and good stability for the analysis of hydrogen peroxide in phosphate buffer solution (pH 7.4). After codeposition of glucose oxidase (GOD) and chitosan (CHIT) coating, the resulting GC/RGO-AuNPs-PB/CHIT-GOD electrode exhibited excellent response to glucose with a sensitivity of $83.6 \text{ mA M}^{-1} \text{ cm}^{-2}$, a low detection limit of $3.0 \text{ }\mu\text{M}$ and a linear range from $3 \text{ }\mu\text{M}$ to 2.0 mM at a detection potential of $+0.1 \text{ V}$ vs. Ag|AgCl reference.

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Enzymatic bioelectrodes for Glucose Biofuel Cells Implanted in animal body

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Electronic medical devices have become an indispensable component of modern healthcare. Currently, a wide variety of THESE devices are being used to monitor physiological parameters of the body, perform therapy and supplement or even entirely replace complex biological functions. Cardiac pacemakers, cardioverter-defibrillators neuro-stimulators, drug delivery chip and cochlear implants are some examples. The life times of these implantables devices is directly depending from batteries used as power supply. Although such batteries continue to be considered as the first choice in supplying power to electronic medical implants, there are numerous efforts to develop alternative power-supply systems that are capable of operating independently over prolonged periods of time without the need of external recharging or refuelling. Several tracks are being explored in order to power implanted devices with energy scavenged from human body. However, systems that take advantage of Seebeck thermoelectric effect, vibrations or body movements to scavenge power from an implanted device are limited to about $100\mu\text{W}$ in the best cases due to constraints inherent to human physiology. Glucose Biofuel Cells (GBFC) looks more promising, since glucose is available ubiquitously in body fluids at the reasonably constant level of 5 mmol/L in the Extra-Cellular Fluid. We report exceptional performances obtained with a glucose biofuel (GBFC) implanted in an animal body. This GBFC is based on carbon nanotube/enzyme electrodes, employing glucose oxidase for biocatalytic glucose oxidation and laccase for dioxygen reduction [1]. Once, the GBFC is implanted in abdomen muscle it is able to produce a power output of $38.7\mu\text{W}$, which corresponded to a power density of $193.5\mu\text{W.cm}^{-2}$ and a volumetric power of $161\mu\text{W.mL}^{-1}$ [2]. Moreover we demonstrate that introducing biocompatible polymer matrix inside electrode improves enzyme life times, and enzymatic electrodes preserve their activities inside animal for several months [3]. Moreover we demonstrate that one single implanted enzymatic GBFC can power a light-emitting diode (LED), or a digital thermometer. In addition, no signs of rejection or inflammation were observed after 300 days implantation in the rat.



Single Glucose Biofuel Cells Implanted in Rats Power Electronic Devices

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New approaches to electrochemical estimation of aged distilled beverages antioxidant properties and quality

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Aged distilled beverages (Cognac, Armagnac and other aged brandies) are part of human diet and widely consumed all over the world. They are rich in lignin-derived phenolic antioxidants due to their maturation in wooden barrels. Antioxidant properties are used as parameter characterizing technological aspects of aged distilled beverages production as well as properties of final product. Taking into account the ability of phenolic compounds to participate in electron transfer reactions, electrochemical methods (constant-current coulometry, voltammetry and chronoamperometry) are a good tool for the evaluation of antioxidant properties of alcoholic beverages. Nevertheless, there is no any data about electrochemical behavior of cognacs and brandies at present time.

Stoichiometric coefficients for reactions of cognac antioxidants with coulometric titrants (electrogenerated bromine and hexacyanoferrate(III) ions) have been found for the first time. Ellagic and gallic acids react with both titrants while aldehydes (vanillin, syringic and coniferaldehyde) - with electrogenerated bromine only. Furfurals don't show significant reactivity toward both oxidants. Cognac (11 samples) and brandy (18 samples) total antioxidant capacity (TAC) and ferric reducing power (FRP) based on reactions with electrogenerated bromine and hexacyanoferrate(III) ions, respectively, have been evaluated.

Cognac and brandy components are electrochemically oxidized on multi-walled carbon nanotube modified glassy carbon electrode at 0.44 and 0.59 V in 0.1 M phosphate buffer solution pH 3.0 under conditions of differential pulse voltammetry (DPV). Voltammetric behavior of the main antioxidant constituents of cognac has been investigated. The first signal of cognacs is caused by oxidation of gallic acid as well as syring- and coniferaldehydes. The second peak corresponds to ellagic acid oxidation. DPV approaches for evaluation of cognac and brandy antioxidant capacity (AOC) have been developed.

One-step chronoamperometry at 0.59 V for 75 s has been applied for the cognac and brandy AOC evaluation. Ellagic acid being the main antioxidant of cognac has been used as reference substance. The chronoamperometric response of ellagic acid is linear in the range of 0.66-52.8 μM with the limits of detection and quantification of 0.19 and 0.63 μM , respectively. AOC in ellagic acid equivalents per 100 mL of cognac and brandy for different denominations (11 cognacs and 11 ordinary and vintage brandies) has been estimated.

TAC, FRP and AOC of cognacs and brandies increases with the age of the beverages that is caused by longer aging in wood casks. Positive correlations ($r=0.8311-0.9847$) with common parameters characterizing antioxidant properties of beverages, in particular antiradical activity toward DPPH $^{\bullet}$ and total phenolics content have been observed.

The electrochemical approaches developed have been tested for evaluation of brandies quality using 10 samples 5 of which were recognized as falsification by gas chromatography. Electrochemical data for falsifications are statistically significant differ from brandies. AOC based on chronoamperometric measurements equals to zero for 4 falsifications. Finally, electrochemical methods are characterized by simplicity, cost-efficiency and reliability of results and can be successfully applied for the cognac and brandy quality control.

The financial support of Russian Foundation for Basic Research (grant 14-03-31173) is gratefully acknowledged.

Redox activity monitoring in intact barley aleurone cells during gibberellic acid induced programmed cell death

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Programmed cell death (PCD) has an important role in seed germination process and it is an essential component of plant defence mechanism against pathogens, hence it can influence the outcome of crop plant germination, yield, quality and interactions with pathogens [1, 2]. The cells in barley (*Hordeum vulgare*) aleurone layer play a key role in seed germination processes controlled by phytohormones such as gibberellic acid (GA) and abscisic acid (ABA). Even though evidence exists showing that reactive oxygen species play an important role in PCD and that there are significant changes in redox balance during GA induced PCD [5], the exact mechanisms of these processes are not fully elucidated in plant cells [3, 4].

To obtain a better understanding of redox activity related events during seed germination and PCD, it is important to be able to measure changes induced in intra and extracellular redox activity, preferably without destroying cell integrity.

Traditionally, redox activity in plant cells is evaluated using colorimetric assay by measuring enzyme activity from cell extracts after the cells of interest were isolated and lysed [5]. Electrochemical techniques have been successfully used to evaluate intracellular redox activity in living intact mammalian [6], yeast [7] and bacterial cells [8]. An amperometric detection system for probing intracellular redox activity in whole cells, based on the menadione (M)/ferricyanide double mediator system was developed and applied by Heiskanen et al. [9].

Given the need for redox activity probing in living plant tissues while keeping the cells intact as well as the capability of electrochemical redox activity assays, we propose the development of a non-destructive electrochemical assay for redox activity monitoring during GA-induced PCD in barley aleurone layers.

The method developed in this project allows intra- and extracellular, as well as membrane-associated redox activity measurements from intact plant tissue. A linear correlation was found between increased reducing capacity and number of live cells up to 48 h GA exposure. The increased reducing capacity of the cells during GA exposure is strongly related to their intracellular and/or membrane-related M reducing capacity. The M reducing capacity of the cells suggests the possible involvement of M-reducing flavoenzymes, such as quinone reductases, in GA related PCD.

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